



Comparative Anatomical Studies on *Saponaria glutinosa* M. Bieb. and *Saponaria prostrata* Willd. subsp. *prostrata* (Caryophyllaceae) Endemic to Turkey

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Received : 09/01/2021

Revised : 23/01/2021

Accepted : 10/02/2021

ABSTRACT: Some *Saponaria* species are naturally grown in Turkey. Anatomical, palynological and micromorphological investigations on some *Saponaria* species were carried out by researchers. However, there are very few anatomical studies on *Saponaria* species. In this study, the anatomical structures of *S. glutinosa* and *S. prostrata* subsp. *prostrata* were searched to provide more information for future systematic works. *S. prostrata* subsp. *prostrata* is endemic species to Turkey. *S. glutinosa* and *S. prostrata* subsp. *prostrata* were collected during the flowering period and natural population. Stem, leaf and corolla samples of these plants were fixed in 70% ethyl alcohol to determine the min. and max. values of width-length measurements. The paraffin method using microtomes was applied for the cross sections of stem and leaf. Safranin O and fast green was used for dying. Besides, sartur reagent was applied to the sections for investigation of some anatomical tissues. Stomatal index in adaxial and abaxial surfaces of leaves was also calculated. A superficial-sections from adaxial and abaxial surfaces of leaves were taken to determine stoma types. The anatomical characteristics of stem in *S. glutinosa* was similar to ones of stem in *S. prostrata* subsp. *prostrata*. Druse crystals were seen in stems and leaves of both species. The leaf of *S. glutinosa* is bifacial whereas *S. prostrata* subsp. *prostrata* has ekvifacial leaf type. Diasitic type stoma was also seen in both species. Besides, stoma was seen on both adaxial and abaxial surfaces of leaf in *S. glutinosa* and in *S. prostrata* subsp. *prostrata*. The values of stoma index in adaxial and abaxial surfaces of leaf are higher in *S. prostrata* subsp. *prostrata* than *S. glutinosa*.

Keywords: Anatomy, endemic, *Saponaria glutinosa*, *Saponaria prostrata* subsp. *prostrata*

INTRODUCTION

The Caryophyllaceae family is included roughly 100 genera and 3000 species (Ulukuş, 2018). This family is spread in the northern hemisphere (Hernández-Ledesma et al., 2015). *Saponaria* L. genus belong to Caryophyllaceae family and is included 25 taxa (18 species), of which 12 are endemic to Turkey (Gemici et al., 2011; Güner et al., 2012). Caryophyllaceae family has many economic plants for Turkey. Some species of the family are known as coven, female coven and halva roots are known as fire extinguisher, gold polishing, cleaner and softener of delicate fabrics and crispness giving to halva (Korkmaz and Özçelik, 2011). This family has also ornamental flowering plants like *Dianthus* sp., *Gypsophila* sp., *Saponaria* sp. and *Silene* sp. (Chandra and Rawat, 2015). *Saponaria* species are named “sabunotu” in Turkey, because these species have included “saponin” content which can be used as a soap for cleaning properties. *Saponaria* species are generally used against some illnesses to treat (Duke, 1985). Biological activity of some *Saponaria* species have shown that they are active against some cancer cell lines (Hickie et al., 2011; Koz, 2018). *Saponaria* species include antiproliferative, immune supporting and hemolytic activities as well (Bogoiavlenskii et al., 1999; Balsevich et al., 2012). Some species of *Saponaria* include triterpenoid products and saponins (Ataşlar 2004) used as a soap substitute or for medicinal purposes (Bittrich, 1993; Gemici et al., 2011).

Saponaria species are herbaceous perennials and annuals, some with woody plants. The flowers are abundant, five-petalled and usually in shades of pink or white (Thieret John and Rabeler Richard, 2005; RHS, 2008). *Saponaria glutinosa* M. Bieb. is known as “kargasabunu”, but *Saponaria prostrata* Willd. subsp. *prostrata* is named “ebemterliği” in Turkey. *Saponaria prostrata* subsp. *prostrata* is also endemic species to Turkey (Akpulat, 2018).

Some anatomical features of Caryophyllaceae family and *Saponaria* species were mentioned (Metcalf and Chalk, 1950). Anatomical (Ataşlar, 2004), palynological (Arkan and İnceoğlu, 1992; Gemici et al., 2011) and micromorphological investigations (Çinbilgel et al., 2007) were carried out by other researchers. However, there are very few anatomical studies *Saponaria* species which are naturally grown in Turkey. For this purpose, in this study anatomical structures of *S. glutinosa* and *S. prostrata* subsp. *prostrata* were searched to provide more information for future systematic works.

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MATERIALS AND METHOD

In this research paper, *S. glutinosa* and *S. prostrata* subsp. *prostrata* were used for plant materials. Both *S. glutinosa* and *S. prostrata* subsp. *prostrata* were collected during the flowering period and natural population (Table 1, Figure 1). Their systematic descriptions were carried out according to Davis (Davis, 1982).

Table 1. Collected areas, years and voucher numbers of *S. glutinosa* and *S. prostrata* subsp. *prostrata*

<i>S. glutinosa</i>	<i>S. prostrata</i> subsp. <i>prostrata</i>
1 km NE Küçükçay village	4 km SW of Köşeler village
Degraded forest	Open forest
1100 m	1500 m
2016	2016
Cansaran 1663-A	Cansaran 1289

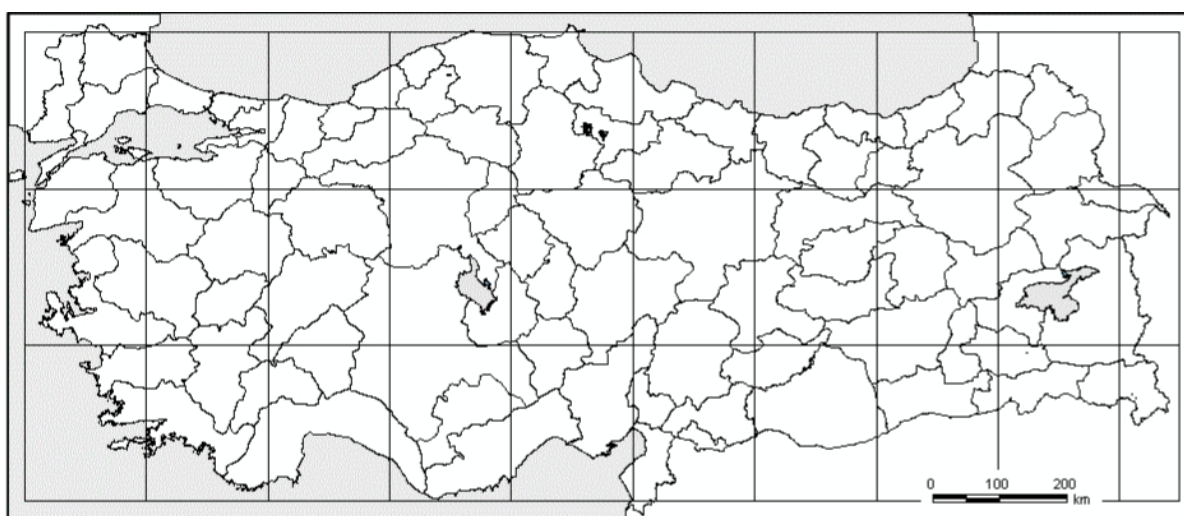


Figure 1. Distribution map of *S. glutinosa* (▼) and *S. prostrata* subsp. *prostrata* (■) in Turkey

■: *S. prostrata* subsp. *prostrata* ▼ : *S. glutinosa*

Stem, leaf and corolla samples of these plants were fixed in 70% ethyl alcohol to determine the minimum and maximum values of width-length measurements in stem, leaf and corolla tissues. The studies were performed on average 30 fresh specimens kept in 70 % alcohol. The paraffin method using microtomes was applied for the cross sections of stem and leaf (Algan, 1981). Safranin O and Fast green was used for dyeing. Besides, safranin reagent was applied to the sections for investigation of some anatomical tissues (stoma and corolla) easily (Çelebioğlu and Baytop, 1949). A Leica ICC50 HD binocular light microscope, a Leica Digital Camera and X10 - X40 objectives were used to measure the minimum and maximum width-length of various tissues in stem, leaf and corolla. Stomatal index in adaxial and abaxial surfaces of leaves was calculated according to Meidner and Mansfield (1969). A superficial-sections from adaxial and abaxial surfaces of leaves were taken to determine stoma types. Preparations were photographed with a Leica ICC50 HD binocular light microscope and a Leica Digital Camera. Anatomical studies of *S. glutinosa* and *S. prostrata* subsp. *prostrata* were carried out in Department of Biology, Faculty of Art and Science, Amasya University.

RESULTS AND DISCUSSION

The study is showed necessary information on anatomy of *S. glutinosa* and endemic *S. prostrata* subsp. *prostrata*. The paper is the first data on anatomy of the species.

In the cross-section of stem, both *S. glutinosa* and *S. prostrata* subsp. *prostrata* have round stem. *S. glutinosa* has 1-layered epidermis, consisting of rectangular cells (15-57.5 x 12.5-35 µm) (Table 2). The upper surface of the stem has nonglandular and glandular hairs. Cortex tissue located under the epidermis is composed 4-5-layered ovoid or rectangular parenchymatic cells (20-50 x 7.5-30 µm). Beneath the cortex, there is an endodermis which is 1-layered ovoid cells. The pericycle has 8-9-

layered schlerenchyma cells (2.5-30 x 10-35 μm) (Figure 2a). The phloem is 4-5-layered cells. Just beneath the phloem, there is the xylem tissue. The pith consists of big round parenchymatous cells with small cells (Figure 2 a). The pith cells of *S. glutinosa* has also druse crystals (Figure 2b). Similarly, *S. prostrata* subsp. *prostrata* has nonglandular and glandular hairs on upper surface of epidermis. *S. prostrata* subsp. *prostrata* has 1-layered epidermis, consisting of rectangular cells (17.5-52.5 x 12.5-27.5 μm) (Table 2). Beneath the epidermis, there is a cortex tissue which is composed ovoid or rectangular parenchymatic cells (10-55 x 10-27.5 μm) (Figure 2c). An endodermis of *S. prostrata* subsp. *prostrata* has 1-layered tissue which is composed of ovoid cells. The pericycle has 5-6-layered schlerenchyma cells (7.5-25 x 7.5-22.5 μm). The phloem is 5-6-layered cells. The pith of *S. glutinosa* has large and small round parenchymatous cells in the centre of the species (Figure 2c). The pith cells of *S. prostrata* subsp. *prostrata* have big druse crystals as well (Figure 1 d). In the study, it was found that the anatomical characteristics of stem in *S. glutinosa* is similar to ones of stem in *S. prostrata* subsp. *prostrata*. These anatomical features belong to stem were described according to Metcalfe and Chalk (1950). The anatomical characteristics of stem in *S. glutinosa* and *S. prostrata* subsp. *prostrata* were also reported in stem of *S. karapinarensis*, *S. kotschyi* and *S. pamphylica* (Ataşlar, 2004; Çinbilgel et al., 2007; Ulukuş, 2018). Ulukuş (2018) and Çinbilgel et al. (2007) were also determined druse crystals in stem of *S. karapinarensis* and *S. pamphylica*. Schweingruber (2007) reported druse crystals which were very frequent in the stem parenchyma cells of the genera *Dianthus*, *Gypsophila*, *Saponaria* and *Silene*. Similarly, this crystals were seen in stem of *S. glutinosa* and in *S. prostrata* subsp. *prostrata*.

Table 2. Anatomical measurements of *S. glutinosa* and *S. prostrata* subsp. *prostrata* (μm)

Parameter	<i>S. glutinosa</i>		<i>S. prostrata</i> subsp. <i>prostrata</i>	
	Width (μm)	Height (μm)	Width (μm)	Height (μm)
	Min.-Max.	Min. Max.	Min. Max.	Min. Max.
Stem				
Epidermis cell	15-57.5	12.5-35	17.5 -52.5	12.5-27.5
Parenchyma cell	20-50	7.5-30	10-55	10-27.5
Endodermis cell	15-35	5-20	7.5-47.5	7.5-25
Pericycle cell	2.5-30	10-35	7.5-25	7.5-22.5
Trachea cell	5-25	5-22.5	7.5-32.5	7.5-30
Pith cell	17.5-80	25-75	10-85	10-75
Leaf				
Adaxial epidermis cell	10-57.5	15-37.5	20-80	28-42.5
Abaxial epidermis cell	15-72.5	15-27.5	2.5-67.5	17.5-37.5
Palisade cell	10-17.5	37.5-62.5	5-10	32.5 -60
Spongy cell	17.5-20	17.5-42.5	-	-
Trachea cell	7.5-15	7.5-15	5-15	4-20
Corolla				
Adaxial epidermis cell	10-30	7.5-20	12.5-37.5	20-25
Abaxial epidermis cell	12.5-50	20-42.5	27.5-90	22.5 -75
Palisade cell	15-30	12.5-25	12.5-25	15-45
Trachea cell	5-12.5	5-12.5	5-12.5	5-10

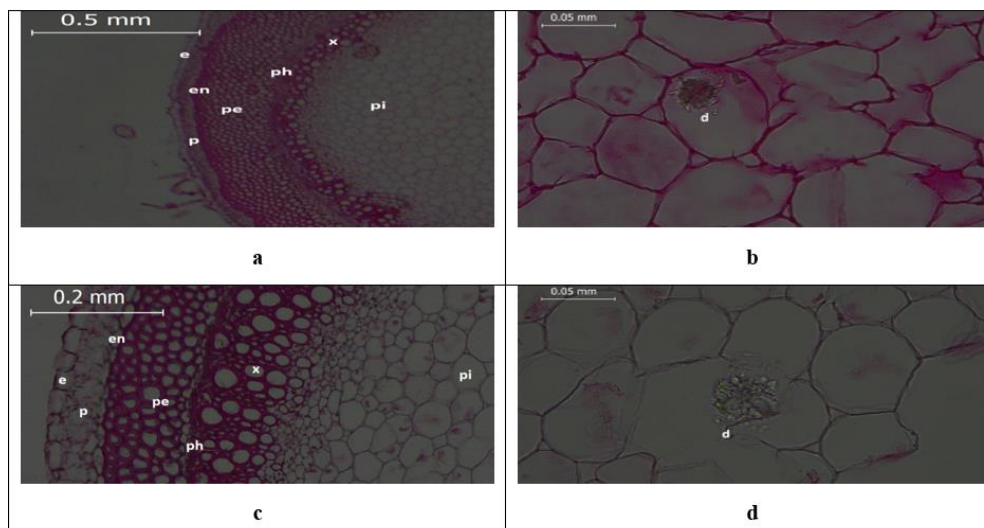


Figure 2. Cross-section of stems in *S. glutinosa* (a, b) and in *S. prostrata* subsp. *prostrata* (c, d). e: epidermis, p: parenchyma, en: endodermis, pe: pericycle, ph: phloem, x: xylem, pi: pith, d: druse crystal in pith cell

On the leaf surfaces of *S. glutinosa* and *S. prostrata* subsp. *prostrata* have nonglandular and glandular hairs on surface of epidermis. Adaxial and abaxial epidermises are covered by a cuticle layer in leaf of *S. glutinosa* species. Both epidermises are 1-layered and consist of oval or rectangular cells ($10-57.5 \times 15-37.5 \mu\text{m}$ / $15-72.5 \times 15-27.5 \mu\text{m}$). The mesophyll is has 2 layer elongated palisade parenchyma cells. Spongy parenchyma has 4-5 layered irregular cells in *S. glutinosa* ($17.5-20 \times 17.5-42.5 \mu\text{m}$) (Figure 3b). The collateral vascular bundle is located in the midrib (Figure 3 a). Besides, the leaf of *S. glutinosa* has druse crystals (Figure 3b). When it comes to the leaf of *S. prostrata* subsp. *prostrata*, both epidermises are covered by a cuticle layer and they are 1-layered oval or rectangular cells. The mesophyll is 3-layer of elongated palisade parenchyma cells and 3-layer lower palisade parenchyma cells ($5-10 \times 32.5-60 \mu\text{m}$) (Figure 3d). There isn't any spongy parenchyma tissue in the leaf of *S. prostrata* subsp. *prostrata*. The collateral vascular bundle is located in the midrib (Figure 3c). Like *S. glutinosa*, the leaf of *S. prostrata* subsp. *prostrata* has druse crystals as well (Figure 3d). In this study, in the cross-section of leaves, the leaf of *S. glutinosa* is bifacial whereas *S. prostrata* subsp. *prostrata* has ekvifacial leaf type (Figure 3b, d). Ekvifacial leaf type was also reported in another *Saponaria* species (Ataşlar, 2004; Çinbilgel et al., 2007; Ulukuş, 2018). Ulukuş (2018), Ataşlar (2004) and Çinbilgel et al. (2007) also mentioned druse crystals in *Saponaria* species leaves. Like these studies mentioned above, these crystals were seen in the leaves of both *S. glutinosa* and *S. prostrata* subsp. *prostrata* species in this paper.

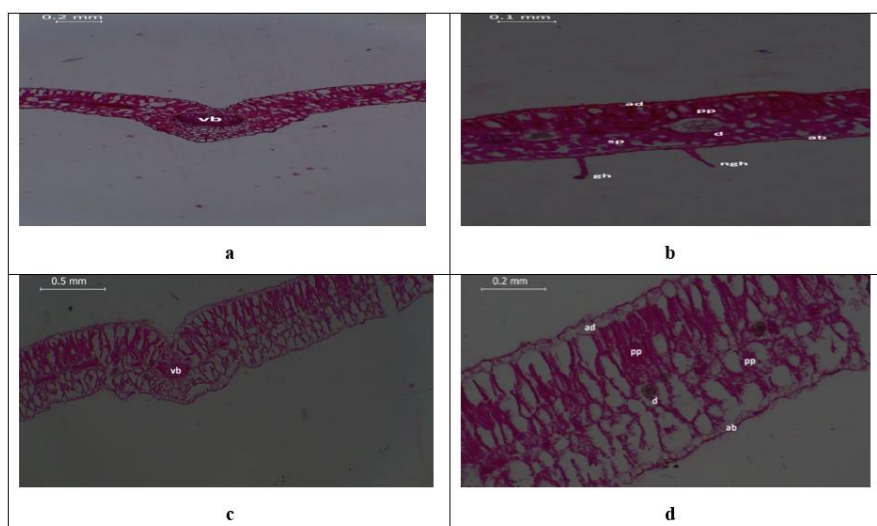


Figure 3. Cross-section of leaves in *S. glutinosa* (a, b) and in *S. prostrata* subsp. *prostrata* (c, d). vb: vascular bundle, ad: adaxial epidermis, pp: palisade parenchyma, sp: spongy parenchyma, ab: abaxial epidermis, d: druse crystal, gh: glandular hair, ngh: nonglandular hair

In the study, stoma index belongs to two species was also studied. The stoma was seen on both adaxial and abaxial surfaces of the leaf in *S. glutinosa* and in *S. prostrata* subsp. *prostrata* (Figure 4a,b,c,d). The values of stoma index in adaxial and abaxial surfaces of leaf are higher in *S. prostrata* subsp. *prostrata* than *S. glutinosa* (Table 3). Both *S. glutinosa* and *S. prostrata* subsp.

prostrata have diasitic type (Caryophyllaceous type) stoma on adaxial and abaxial surfaces (Figure 4a,b,c,d). The difference between both groups was seen on adaxial epidermis cell structure. The adaxial epidermis cells have curved structure in *S. glutinosa* compared to *S. prostrata* subsp. *prostrata* (Figure 4a,c). Diasitic type stoma was seen in *S. kotschyi* and in *S. pamphylica* species (Ataşlar, 2004; Çinbilgel et al., 2007). Besides, diasitic type stoma was also observed in another genus in Caryophyllaceae family such as *Gypsophila lepidioides* Boiss (Özdemir et al., 2010). These anatomical features of the leaves are consistent with those of Metcalfe and Chalk (1950).

Table 3. Stomata index of *S. glutinosa* and *S. prostrata* subsp. *prostrata*

Stomata Index (Mean ± SE)	<i>S. glutinosa</i>	<i>S. prostrata</i> subsp. <i>prostrata</i>
Upper surface	22.187 ± 1.336	32.670 ± 1.000
Lower surface	21.630 ± 1.099	25.868 ± 1.857

*SE: Standart Error

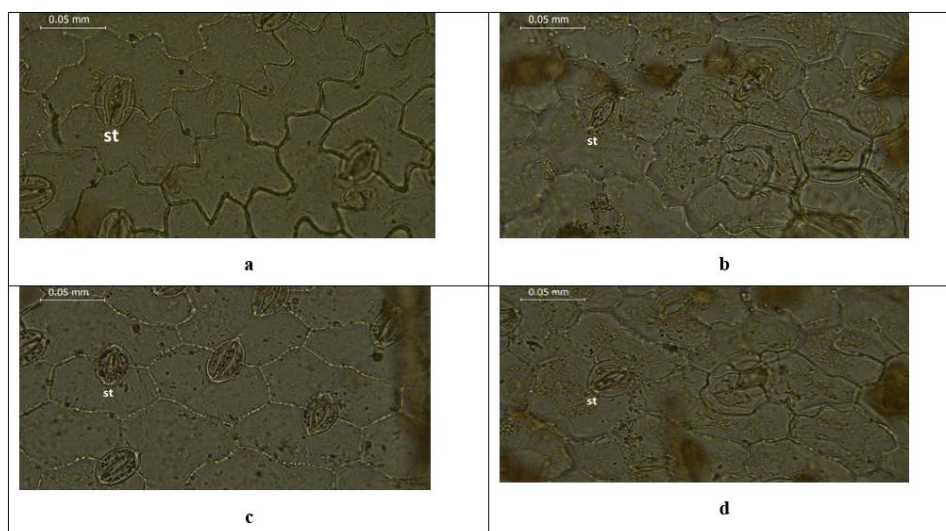


Figure 4. Superficial-section of leaves in *S. glutinosa* (a, b) and in *S. prostrata* subsp. *prostrata* (c, d). vb: vascular bundle, st: stomata

In the cross-section of corolla, cuticle is seen on abaxial and adaxial epidermis cells covered by glandular and nonglandular hairs in both species. Cuticle is present on both adaxial and abaxial epidermis cells in *S. glutinosa* and *S. prostrata* subsp. *prostrata*. Beneath the adaxial epidermis, there is the parenchyma cells with intercellular spaces (Figure 5a,b). Parenchyma cells are 2-3-layered in *S. glutinosa* (15-30 x 12.5-25 µm), but they are 4-5-layered in *S. prostrata* subsp. *prostrata* (12.5-25 x 15-45 µm). Besides, there is the vascular bundle in the midrib in both species (Figure 5a,b).

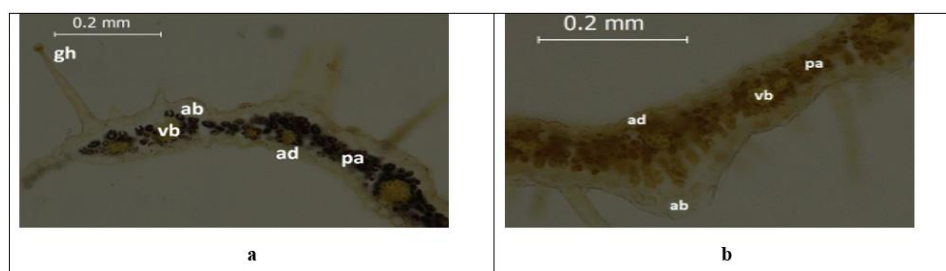


Figure 5. Cross-section of corolla in *S. glutinosa* (a) and in *S. prostrata* subsp. *prostrata* (b). vb: vascular bundle, ad: adaxial epidermis, pa: parenchyma, ab: abaxial epidermis

CONCLUSION

In conclusion, the anatomical characteristics of stem in *S. glutinosa* was similar to ones of stem in *S. prostrata* subsp. *prostrata*. However, the leaf of *S. glutinosa* is bifacial whereas *S. prostrata* subsp. *prostrata* has ekvifacial leaf type. Druse

crystals were seen in stems and leaves of both species. Diasitic type stoma was also seen in both species. Besides, stoma was seen on both adaxial and abaxial surfaces of leaf in *S. glutinosa* and in *S. prostrata* subsp. *prostrata*. The values of stoma index in adaxial and abaxial surfaces of leaf are higher in *S. prostrata* subsp. *prostrata* than *S. glutinosa*. It was thought that anatomical characteristics of *S. glutinosa* and *S. prostrata* subsp. *prostrata* could provide useful information to future systematic works of these species. Besides, the comparative anatomical studies of *S. glutinosa* and *S. prostrata* subsp. *prostrata* present the first data available in the literature.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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