ANATOMICAL DIFFERENCES OF THE TURKISH *STUCKENIA* BÖRNER (POTAMOGETONACEAE) AND THEIR TAXONOMIC SIGNIFICANCE

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Abstract

Anatomical studies of the *Stuckenia* species occurring in Turkey were conducted. The results showed that the presence or absence of interlacunar bundles and the stelar type were the most important in plant anatomical characters of the stem. The leaves of the all investigated species typically had uniseriate epidermis with a thin cuticle, aerenchyma was composed of arm-shaped chlorophyllous cells, similar to those found in the stem and the peduncle. Two lateral vascular bundles and a central vascular bundle are present in the leaves of all investigated species. However, the number of lateral vascular bundles can vary in *S. pectinata* according to leaf width and the fiber bundles are also present in the triquetrous leaves of this species.

Key words: Anatomy, Potamogetonaceae, Stuckenia, Taxonomy, Turkey.

Introduction

Potamogetonaceae is one of the most diverse families of aquatic plants, containing markedly pronounced taxonomic challenges (Wiebleg & Kaplan, 1998; Kaplan et al., 2009). The family Potamogetonaceae is represented by two genera, Potamogeton L. and Groenlandia Gay. The genus Potamogeton is divided into two subgenera; subg. Potamogeton and subg. Coleogeton Reichenbach in Flora of Turkey and East Aegean Islands (Uotila, 1984). Les and Haynes (1996) elevated Potamogeton subg. Coleogeton to generic status, assigning the name Coleogeton (Reichenbach) Les & Haynes. Börner (1912) distinguished the same group as distinct genus Stuckenia (Kaplan, 2008). This fact was overlooked by Les & Haynes (1996) and thus a superfluous name for Stuckenia was introduced. Nowadays, the family Zannichelliaceae is treated as a synonym of Potamogetonaceae. Hence, the genera Althenia Petit and Zannichellia L. previously placed in Zannichelliaceae are transferred into the family Potamogetonaceae. Ultimately, Potamogetonaceae is now represented in Turkey by 5 genera: Althenia (1 species), Groenlandia (monotypic), Potamogeton (12 species, 1 hybrid), Stuckenia (3 species) and Zannichellia (1 species) and 19 taxa in Turkey (Aykurt, 2012).

Stuckenia is characterized mainly by the presence of long leaf sheaths, characteristic leaf and peduncle anatomy and a higher ploidy level (hexaploidy) than in *Potamogeton* s. str. (generally diploids or tetraploids) (e.g. Les & Haynes, 1996; Holub, 1997; Haynes *et al.*, 1998). Kaplan (2008) reported that globally the genus is represented by 7 species and 3 confirmed hybrids, and that the centre of diversity is in the mountains of Central Asia and the adjacent lowlands of Siberia and Kazakhstan, where six species of *Stuckenia* occur. The remaining species, *S. striata* (Ruiz & Pav.) Holub occurs in North and South America. The lowest diversity of the genus is found in Africa and Australia, where this genus is represented by a single and the only cosmopolitan species, *S. pectinata* (L.) Börner (Kaplan, 2008). Three species of this genus, *S. amblyphylla* (C.A.Mey) Holub, *S. filiformis* (Pers.) Börner and *S. pectinata*, occur in Turkey, *S. pectinata* is distributed in every region in Turkey, *S. amblyphylla* is in Erzurum-Kars province, and *S. filiformis* is known only in Antalya Province of Turkey (Uotila, 1984; Aykurt, 2012).

All *Stuckenia* species have only submerged leaves. Stomata are rare and nonfunctional (Haynes *et al.*, 1998). The cuticle is thin or nearly absent, and that the mesophyll varies from 1 to 4 or 5 cell layers, but the palisade is not developed in the submerged leaves of *Potamogeton*. Lacunae are common along the midvein and sometimes occur in the mesophyll. All cells of *Potamogeton* leaves, except those associated with the veins contain chlorophyll. For the genus *Stuckenia*, the structure of the leaf's sheath is the most reliable vegetative characteristic for identification.

In the family Potamogetonaceae, the vascular system is divided into two, cortical and stelar (Tomlinson, 1982; Behnke, 1998). The stem of Potamogeton (s.l.) is characterized by a central stelar vascular system and abundant phloem (Behnke, 1998). The stem's anatomical characters also contain useful information with respect to the taxonomic arrangement of the species, and are also helpful in the identification of fragmentary herbarium specimens (Hagström, 1916; Tur, 1982; Wiegleb, 1990). The diagnostic anatomical characters of stem for Potamogeton (s.l.) were indicated by Wiegleb & Kaplan (1998) as: 1. Stelar type; 2. Endodermis type (which is either of U or 0type, or intermediate U-0-type); 3. Presence of interlacunar bundles; 4. Presence of subepidermal bundles; 5. Presence of the pseudohypodermis. Similar characteristics are used for the peduncle anatomy in this study such as the type of stele and the endodermis, and presence of the pseudohypodermis. In this study, the comparative anatomy of the Stuckenia species of Turkey was examined and the results were compared with previous studies.

Materials and Methods

Plant samples: Specimens were collected in 2014 during field trips in Turkey. Numerous specimens of *S. pectinata* were collected from different areas and habitats, and the leaf shape of this species was observed morphologically as narrowly to broadly linear or triquetrous. For determination of the *S. pectinata*'s leaf anatomy, individuals exhibiting different and extreme morphological features, and those that grow in different habitats were chosen. *S. amblyphylla* was collected from two different localities in East Anatolia and *S. filiformis* was collected from a single location in Antalya Province. The information of the voucher specimens is given in Table 1.

Anatomical investigations: Live samples were fixed in 1:1:1 mixture of ethyl alcohol, glycerin, distilled water and stored in falcon tubes. Short pieces of peduncle and leaf from the middle, and stem from the middle of the internode region of the main stem were cut. Transverse sections \pm 0.05 mm thin were cut by hand using a razor blade. This was done under a stereomicroscope using reflected light and the sections were stained in a drop of water with toluidine blue. After 1–3 minutes, depending on stainability, the sections were washed in distilled water and studied under a light microscope at a magnification 10–40× (general anatomical pattern) or 100× (shape of endodermal cells, occurrence of interlacunar and subepidermal bundles, development of pseudohypodermis).

A detailed review of the use of the stem anatomy in the systematics and identification of species of *Potamogeton* (s.l) is provided by Wiegleb (1990). Terminology used in this study is that of Wiegleb & Kaplan (1998).

Result and Discussion

Stem anatomy: The transverse section of the stem is rounded in all investigated species. The epidermis exhibits rectangular-shaped cells with chloroplasts and a thickened outer periclinal wall, with occasional epicuticular striations. The cortex is composed of a single layered pseudohypodermis with chloroplasts, and aerenchyma with the cells arranged in a honeycomb form (Fig. 1c, f, i). Subepidermal bundles are absent in all investigated species. The aerenchyma lacunae formed by arm-shaped cells have chloroplasts which extend to the epidermis (Fig. 1d, g). Interlacunar bundles also appear scattered among the parenchyma cells of the aerenchyma (Fig. 1c, d, f, i). The total number of interlacunar bundles varies within the species: 5-circle in S. amblyphylla, 2-circle in S. filiformis and 8-circle in S. pectinata. The shape of the endodermal cells is O-type in S. filiformis, U-type in S. amblyphylla and S. pectinata (Fig. 1a, b, e, h). The stele is a four bundle type in the transverse section of S. amblyphylla, with four vascular bundles: four large lacunae of protoxylem, large phloem sieve elements and are partially surrounded by fibers (Fig. 1a, b). The circular or one bundle type is in S. filiformis and S. pectinata. Vascular bundles in S. filiformis and S. pectinata exhibit one central large lacunae of protoxylem and large phloem sieve elements. The lacunae of protoxylem and phloem sieve elements of S. pectinata are larger than S. filiformis's (Fig. 1e, h). The stem anatomical characteristics of the three species investigated in the present study are compared with S. pamirica (Baagöe) Z. Kaplan (as P. recurvatus), S. striata (Ruiz & Pav.) Holub (as P. striatus) and S. vaginata (Turcz.) Holub (as P. vaginatus) studied by Wiegleb & Kaplan (1998) previously. The stem anatomical characteristics of all six species are given in Table 2.

 Table 1. Locality data of collected Stuckenia species for the current study.

Species	Locality	Collection data C. Aykurt (4184) & İ.G. Deniz.	
S. amblyphylla	A9 Ardahan: Ölçek, between Ardahan and Damal, Ölçek River, 1744 m, 38T 320121 4556079, 26.viii.2014.		
	B7 Erzincan: Çayırlı, 2 km from Ozanlı to Yeşilyayla, stream, 2167 m, 37S 582519 4409595, 24.viii.2014.	<i>C. Aykurt</i> (4160) & <i>İ.G. Deniz.</i>	
S. filiformis	C4 Antalya: Gündoğmuş, Ak Mountain, Eğri Lake, 2071 m, 36S 428855 4088125, 17.vii.2014.	<i>C. Aykurt</i> (4130) & <i>İ.G. Deniz.</i>	
	C4 Antalya: Gündoğmuş, Ak Mountain, Eğri Lake, 2069 m, 36S 0249131 4087659, 17.vii.2014.	<i>C. Aykurt</i> (4134) & <i>İ.G. Deniz.</i>	
S. pectinata	C2 Muğla: Köyceğiz, Köyceğiz Lake, 2 m, 27.vi.2014.	<i>C. Aykurt</i> (4040) & <i>İ.G. Deniz.</i>	
	C3 Antalya: Döşemealtı, 3 km to Kırkgöz, channels, 292 m, 36S 285637 41080607, 21.vii.2014.	<i>C. Aykurt</i> (4149) & <i>İ.G. Deniz.</i>	
	C5 Mersin: Gülnar, Sipahili, Babadil River, under the Babadil Bridge, 8 m, 36S 541750 4001970, 30.v.2014.	<i>C. Aykurt</i> (3979) & <i>İ.G. Deniz.</i>	

Table 2. The comparison of the stem anatomical characteristics of six <i>Stuckenia</i> species.								
Characters	S. amblyphylla	S. filiformis	S. pectinata	<i>S. pamirica</i> (Wiegleb and Kaplan, 1998)	<i>S. striata</i> (Wiegleb and Kaplan, 1998)	<i>S. vaginata</i> (Wiegleb and Kaplan, 1998)		
Stelar type	Four bundle	Circular	Circular	Four bundle	Complex oblong or rarely circular	Trio- or four bundles		
Endodermis type	U-type	O-type	U-type	U-type	U-type, rarely O-type	U-type		
Number of interlacunar bundle	5 circles	2 circles	8 circles	1-2 circles	1-2 circles	(3–)4 circles		
Subepidermal bundle	Absent	Absent	Absent	Present	Absent	Absent, rarely a few present		
Cell-layer of pseudo-hypodermis	1-layered	1-layered	1-layered	1-layered	1(-2) layered	1-2 layered		



Fig. 1. The stem transversal sections of *S. amblyphylla* (a, b, c), *S. filiformis* (d, e, f) and *S. pectinata* (g, h, i) (ar: aerenchyma, cu: cuticle, en: endodermis, ep: epidermis, fb: fiber bundle, ilb: interlacunar bundle, ph: phloem, phy: pseudohypodermis, px: protoxylem, st: sieve tube element).

Peduncle anatomy: In all investigated species, the shape of the transverse section of the peduncle is rounded (Fig. 2a, e, i). The epidermis exhibits rectangular-shaped cells with chloroplasts and a thickened outer periclinal wall, with occasional epicuticular striations in S. amblyphylla and S. filiformis (Fig. 2b, h). The surface of cuticle is distinctly papillose in S. pectinata (Fig. 2j). 1-layered pseudohypodermis with chloroplasts in the cortex and aerenchyma with the cells arranged in a honeycomb form is common to all three species (Fig. 2b, h, j). In all investigated species, the subepidermal- and interlacunar bundels are absent. The aerenchyma lacunae formed by arm-shaped cells have chloroplasts that extend to the epidermis (Fig. 2b, f, j). In investigated species, the shape of the endodermal cells is U-type (Fig. 2d, g, l). The stele is oblong type in the transverse section in all the species (Fig. 2c, g, k).

Leaf anatomy: The epidermis of all investigated specimens is uniseriate and chlorophyllous, with a thin cuticle layer. The mesophyll is formed by aerenchyma. Similar to those found in the stem and peduncle, the leaf aerenchyma is composed of arm-shaped chlorophyllous cells. All three species have complete vascular bundles at laterals to the epidermis. The vascular bundles are collateral and have a fiber cap in the periphery (Fig. 3b, c, f and Fig. 4b, c, g).

The specimens of *S. amblyphylla* were collected from two different locations, leaves are elliptical in transversal section. There are two lateral vascular bundles except the midrib and 10 lacunae, two of which are larger around the midrib (Fig. 3a).

S. filiformis is known only from one location in Turkey. The leaves of this species are rounded to elliptic in transverse section (Fig. 3d). There are two large lacunae along the midrib and small lacunae in the mesophyll. Two lateral vascular bundles are also present, resembling *S. amblyphylla* (Fig. 3e).



Fig. 2. The peduncle transversal sections of *S. amblyphylla* (a, b, c, d), *S. filiformis* (e, f, g, h) and *S. pectinata* (i, j, k, l) (ar: aerenchyma, cu: cuticle, en: endodermis, ep: epidermis, fb: fiber bundle, ph: phloem, phy: pseudohypodermis, px: protoxylem).



Fig. 3. The leaf transversal sections of *S. amblyphylla* (a, b, c) and *S. filiformis* (d, e, f) (ed: epidermis, fb: fiber bundle, la: lacunae, lv: lateral vein, mv: midvein, ph: phloem, px: protoxylem).

The S. pectinata specimen collected from a river (Aykurt 3979), is elliptic in transversal section and two vascular bundles occur at the lateral edges except at the midrib. The lacunae encircle the midrib, and two of them are distinctly larger than the others. Totally 5-6 lacunae are present around the midrib, and one in the mesophyll is observed in this specimen (Fig. 4a). The leaf width of the specimen, collected from standing water in a canal (Aykurt 4149), is significantly larger than all other collected S. pectinata specimens, and it is also elliptic in the transverse section. Unlike the others, there are four lateral vascular bundles, and 6 large lacunae around the midrib, of which the two on the lateral edges, are clearly larger. Small lacunae are also present in the mesophyll (Fig. 4d). The triquetrous leaves of the S. pectinata collected from a lake (Aykurt 4040) show differences from the others in transversal section, with the presence of fiber bundles under the epidermis at the two lateral edges (Fig. 4f). There are 2 large and 3-4 small lacunae located around the midrib, and two lateral vascular bundles are found at the lateral edges (Fig. 4e).

The transverse section of the leaf sheath is known to be an important characteristic for the identification of the *Stuckenia* species. The leaf sheath is closed in *S. amblyphylla* and plicate in *S. filiformis*, whereas it is open in *S. pectinata*. The important leaf and peduncle anatomical characters of *S. amblyphylla*, *S. filiformis* and *S. pectinata* occuring in Turkey are given in Table 3.

The genus *Stuckenia* has seven species (Kaplan, 2008). Three species of the genus occuring in Turkey were anatomically evaluated. The stem anatomical characters of *Stuckenia* species were examined by Wiegleb & Kaplan (1998). The stem anatomy of *S. amblyphylla, S. filiformis* and *S. pectinata*, the species investigated in this study, are compared with the results of Wiegleb & Kaplan (1998), our results show differences, particularly with respect to the stelar type and number of the interlacunar bundles. Looking as stem anatomy; the stelar type, the occurrence of the subepidermal and the interalcunar bundles, their number and size are important characteristics for the identification of the *Stuckenia* species (Wiegleb, 1990; Wiegleb & Kaplan, 1998). If all the *Stuckenia* species except *S. macrocarpa*, which occurs in Russia and Kazakhstan, are evaluated worldwide, the subepidermal bundles which are present in *S. pamirica* and are rarely few in *S. vaginata*, however, according to results of Wiegleb & Kaplan (1998) they are not present in *S. striata*. Subepidermal bundles are absent in *S. amblyphylla*, *S. filiformis* and *S. pectinata*. Accordingly, the presence or absence of subepidermal bundles is significant for the identification of *Stuckenia* species and hybrids.

Wiegleb & Kaplan (1998) reported, four vascular bundles which were rarely oblong type in the stele of S. filiformis, but we observed circular type of vascular bundles in stem transverse section. This situation indicates that the stelar type may be high by variable for the species. Also, the number of interlacunar bundles can be quite variable within each species. We observed that in S. pectinata, the number of the interlacunar bundles were 8, but Wiegleb & Kaplan (1998) reported it as a single more or less complete circle for the same species. Similarly the number of the interlacunar bundles is 5 in S. filiformis, but they were observed as 1(-3) incomplete circles by Wiegleb & Kaplan (1998). Although we determined 2 interlacunar bundles in S. amblyphlla where as Wiegleb and Kaplan (1998), identified 1 incomplete circle in the same species.

S. pectinata is the most widespread species of the genus, occurring on all continents of the world except Antarctica and it is also the most frequent of the Stuckenia species encountered in Asia (Kaplan, 2008). This species is known to be one of the most variable species of Potamogetonaceae. The leaf anatomy of S. pectinata has been studied on the basis of the specimens collected from three different localities in this study. As findings of the anatomical investigation of these specimens, the leaves are elliptic to triquetrous in transversal sections and typically two completeness vascular bundles are present at the laterals. As leaf width expands, the number of the lateral vascular bundles may increase. Typically, two large and four small lacunae present around the midrib, but the number of the small lacunae can vary according to the maturity of the leaves. Occasionally the leaves may be triquetrous, and the fiber bundles are observed under the epidermis at the two lateral edges of triquetrous leaves. The elliptical shape in transversal section of S. amblyphylla's leaves resembles that of S. pectinata, but there are more lacunae around the midrib. S. filiformis has rounded to elliptic leaves in transverse section, and two large air lacunae also present along the midrib. According to the results of the leaf anatomy study, the number of lacunae along the midrib is the most important diagnostic character for identification of the species investigated by the present study.



Fig. 4. The leaf transversal section of *S. pectinata*: vouchered specimens of *C.Aykurt* 3979 (a, b, c), *C. Aykurt* 4149 (d) and *C. Aykurt* 4040 (e, f, g) (cu: cuticle, ep: epidermis, fb: fiber bundle, la: lacunae, lv: lateral vein, mv: midvein, ph: phloem, px: protoxylem).

Characters	S. amblyphylla	S. filiformis	S. pectinata	
Leaf anatomy				
Leaf shape in transversal section	elliptic	rounded to elliptic	elliptic to triquetrous	
Lateral vascular bundles (number)	2 2		2 or 4	
Lacunae along midrib (number)	10 2		5–6	
Fiber bundles	absent	absent	present in the triquetrous leaves	
Leaf sheath type	closed	plicate	open	
Peduncle anatomy				
Stelar type	Oblong type	Oblong type	Oblong type	
Endodermis type	U-type	U-type	U-type	
Cell-layer of pseudohypodermis	1-layered	1-layered	1-layered	

 Table 3. The comparative leaf and peduncle anatomical characteristics of S. amblyphylla,

 S. filiformis and S. pectinata as they occur in Turkey.

Appendix. Additional specimens collected and examined

Stuckenia pectinata: A2 Sakarya: New Eşme environs, Sapanca Lake, 30 m, 01.07.2014, 36T 0265004 4512730, C.Avkurt (4086) & İ.G.Deniz. Bursa: İznik, City Centre, İznik Lake, Yalova road, 93 m, 02.07.2014, 35T 730488 4479740, C.Aykurt (4099) & İ.G.Deniz. Bursa: Between İznik and Yalova, İznik Lake, 91 m, 02.07.2014, 35T 729560 4477279, C.Aykurt (4100) & I.G.Deniz. A3 Bolu: Abant Lake, 1339 m, 30.06.2014, 36T 0353918 4495988, C.Aykurt (4066) & İ.G.Deniz. Sakarya: Karasu, Kamışlı lake located North side of Sülüklü Lake, 4 m, 01.07.2014, 36T 0279086 4561150, in lake, C.Aykurt (4082) & İ.G.Deniz. A7 Sivas: İmranlı, İmranlı Dam, 1646 m, 28.08.2014, 37S 433841 4412864, C.Aykurt (4203) & İ.G.Deniz. A8 Erzurum: Uzundere, Tortum Lake, 1018 m, 25.08.2014, 37T 724849 4504156, C.Aykurt (4165) & İ.G.Deniz. A9 Kars: Çıldır, Çıldır River, 1900 m, 27.08.2014, 38T 343483 4555011, C.Aykurt (4186) & İ.G.Deniz. Kars: Çıldır Lake, 1976 m, 27.08.2104, 38T 346848 4550067, C.Aykurt (4191) & İ.G.Deniz. Kars: Arpaçay, Çanaksu village environs, Çıldır Lake, 1970 m, 28.08.2014, 38T 355730 4534376, C.Aykurt (4194) & İ.G.Deniz. Kars: Susuz, Gölbaşı village, Aygır Lake, 2130 m, 28.08.2014, 38T 330410 4514881, C.Aykurt (4197) & İ.G.Deniz. Kars: Susuz, 20 km to Kars, Çamçavuş stream, under Çamçavuş Bridge, 1680 m, 28.08.2014, C.Aykurt (4198) & I.G.Deniz. Kars: Kars to Erzurum, Paşaçayır stream, 1744 m, 28.08.2014, 38T 336033 4495518, C.Aykurt (4202) & İ.G.Deniz. B2 Denizli: Çivril, across Beydilli village, Işıklı Lake, 850 m, 04.07.2014, C.Aykurt (4104) & İ.G.Deniz. Denizi: Çivril, Gökgöl, wetlands, 836 m, 04.07.2014, 36 S 241557 4232842, C.Aykurt (4109) & İ.G.Deniz. C2 Muğla: Köyceğiz, city centre, Köyceğiz Lake, 2-3 m, 27.06.2014, C.Aykurt (4040) & İ.G.Deniz. Muğla: Ortaca, Dalyan channel, 3-5 m, 27.06.2014, C.Aykurt (4043) & I.G.Deniz. Muğla: Ula, Akyaka, Kadın Azmağı, river, 3-5 m, 27.06.2014, C.Aykurt (4044) & İ.G.Deniz. C3 Antalya: Antalya to Serik, Düden river, under antiquity bridge, Cırnık Suyu, 50 m, 27.05.2014, C.Aykurt (3970) & İ.G.Deniz. Antalya:

Aksu, under Tehnelli bridge, Tehnelli river, 25.05.2014, C.Aykurt (3974) & İ.G.Deniz. Antalya: Manavgat, Taşkesiği, pond, 400 m, 18.07.2014, C.Aykurt (4138) & İ.G.Deniz. Antalya: Manavgat, Manavgat river, 19.07.2014, C.Aykurt (4146) & İ.G.Deniz. Burdur: Bucak, between Bucak and Ağlasun, across Kibrit village, wetlands, 14.08.2012, C.Aykurt (3426) & İ.G.Deniz. Burdur: Burdur: Bucak, Alkaya village, across Onaç dam, wetlands, 15.07.2014, C.Aykurt (4125) & İ.G.Deniz. Konya: Beyşehir, Beyşehir Lake, under the bridge, channel, 1140 m, 18.07.2014, C.Aykurt (4142) & İ.G.Deniz. Konya: Beyşehir, Beyşehir Lake, 1130 m, 19.07.2014, C.Aykurt (4145) & İ.G. Deniz. C5 Mersin: Silifke, Silifke-Akgöl road, 6 km to Dalyan, Göksu channel, 30.05.2014, C.Aykurt (3982) & İ.G. Deniz. Osmaniye: Kadirli, Bahçe village, Kastabala Valley, Yeniköy Wetlands, 54 m, 31.05.2014, 37S 0248261 4115815, C. Aykurt (3989) & I.G.Deniz (Akdeniz University Herbarium).

Conclusions

The stem, peduncle and leaf anatomy of the Turkish species of Stuckenia were evaluated. The presence or absence of interlacunar bundles and the stelar type are the most important characteristics of the stem anatomy. However, these characteristics can vary within different individuals of the same species when the results of the stem anatomy were compared with previous studies. The peduncle anatomy of the investigated species are quite similar to one another. The leaves of all investigated species typically have uniseriate epidermis with thin cuticle. aerenchvma composed of arm-shaped chlorophyllous cells, similar to those found in the stem and peduncle. Two lateral vascular bundles and a central vascular bundles are present in the leaves of all species investigated. However, the number of lateral vascular bundles can vary in S. pectinata according to the leaf width and fiber bundles also present in the triquetrous leaves of this species. The data obtained from the leaf anatomy shows that the number of lacunae around midrib are useful characters for identification of the Stuckenia species investigated in this study.

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