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ARTICLE

Epidermal micromorphology of floret parts in *Aeluropus* (Poaceae)

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ABSTRACT *Aeluropus* from Poaceae comprises 5 species in the world and 3 species in Iran. This halophytic perennial is distributed in salty and dry soils of Asia, Europe, and Africa. In addition to being used as fodder, it can stabilize the soil by its rhizome or stolon. These features make *Aeluropus* a valuable plant. In this study, lemma and palea of 10 populations of *Aeluropus* were studied micromorphologically by scanning electron microscope (SEM) to determine diagnostic features among species studied. Eight characters as micro-prickle, macro-hair, long cell outline, cork and silica cells, papilla, salt gland, and epicuticular wax were studied. The occurrence of salt glands and silica cells in populations/taxa studied showed the ability of *Aeluropus* to tolerate harsh habitats. Our result showed the taxonomic value of floret micromorphological features to separate *Aeluropus* species.

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Introduction

Aeluropus Trin. (Poaceae, Chloridoideae) is one of the two genera of subtribe Aeluropodinae with 5 species in the world (The Plant List 2013). Previously it comprised 4 species in Iran (Bor 1970). As *A. pungens* (M. Bieb.) K. Koch was synonymized to *A. littoralis* (Gouan) Parl., its species reduced to three taxa (The Plant List 2013). Some infra-specific taxa were recorded for this genus in Iran (Khodashenas 2009).

Members of this halophytic weed are distributed in tropical regions of Asia, Europe, and Africa (Zhang et al. 2006). This genus contains rhizomatous or stoloniferous perennials that help the soil stabilization. It has dense capitate, spike like or panicle inflorescence with compressed spikletes, chartaceous unequal glumes which are shorter than lemma, keeled lemma which is glabrous or hairy on margins, palea with ciliate on keel and oblong-elliptic or oblong-obovate caryopsis (Bor 1970; Zhang et al. 2006).

Aeluropus is distributed in saline soils, sandy shores and dry regions (Zhang et al. 2006). Members of Poaceae family grown in saline soils, have unicellular or bicellular salt glands as a common adaptive feature (Wahit 2003; Kobayashi 2008; Céccoli et al. 2015). All Poaceae subfamilies have micro-hairs except Pooideae (Amarasinghe and Watson 1988, 1989; Kobayashi 2008) but functional salt glands are only observed in Chloridoideae (Bell and Ó Leary 2003; Kobayashi et al. 2007; Hameed et al. 2013). This subfamily is adapted to harsh and saline environments by having salt gland (Taleisnik and Anton 1988; Columbus et al. 2007; Peterson et al. 2010).

The importance of micromorphological characters in Poaceae has been established by different authors. Different vegetative and reproductive parts as leaf (Mavi et al. 2011; Ortúňez and Cano-Ruiz 2013), glume (Klimko and Wysakowska 2015), lemma (Acedo and Llamas 2001; Li et al. 2010, Ortúňez and Cano-Ruiz 2013; Harms and Mendenhall 2015), palea (Klimko et al. 2009; Ortúňez and De La Fuente 2010), and caryopsis (Terrell and Peterson 1993; Gandhi et al. 2013, Zhang et al. 2014; Liu et al. 2015) have been studied micromorphologically to solve taxonomic problems at different levels in Poaceae.

Lemma and palea epidermal characters are of taxonomic value in identifying and studying relationship between genera and species in Chloridoideae (Liu et al. 2010). There are limited studies on the micromorphology of *Aeluropus* species. Liu et al. (2010) studied lemma and palea characters in *A. littoralis*. Features as long cell outline, cork cell, micro-hair, macro-hair, papillae, micro-prickle and silica body have been used to characterize this taxon from other members of Chloridoideae.

This study aims to describe micromorphological characters of lemma and palea in *Aeluropus* species in Iran and to discuss taxonomic value of these features at interspecific level.

Table 1. Voucher details of population studied.

Species	No.	Locality	Longitude	Latitude	Altitude (m)	Voucher No.
A. littoralis (Gouan) Parl.	1	Kerman, Kahnooj	28º 01' 15''	57º 43' 33''	531	99 a ALUH
	2	Sistan and Baluchestan, Hirmand, Bar-Ahuyi	31º 06' 46''	61º 47' 01''	481	ha-169 ALUH
	3	East Azerbaijan, Tabriz to Ahar, Talkheh Rud	38˚ 01' 55''	46 º 56' 58''	1635	8511 ALUH
	4	Tehran, Saveh	35º 01' 17''	50º 21' 24''	998	8512 ALUH
A. lagopoides (L.) Thwaites	5	Semnan, Garmsar	35º 13' 06''	52º 20' 27''	850	8513 ALUH
	6	Kerman, Jazmourian, Zeh-e-kalut	27º 48' 15''	58º 36' 11''	392	99 b ALUH
	7	Sistan and Baluchestan, Hirmand, Bar-Ahuyi	31º 06' 46''	61º 47' 01''	481	ha-168 ALUH
	8	Fars, Maharlu lake	29º 26' 05''	52º 46' 38''	1461	66947 ALUH
A. macrostachyus Hack.	9	Kerman, Kahnooj	28º 01' 15''	57º 43' 33''	531	851 ALUH
	10	Sistan and Baluchestan, 20 km of Mirjaveh to Jaleq	27º 35' 33''	62º 41' 28''	849	49878 IRAN

Materials and Methods

To study the micromorphological features, 10 accessions of 3 species of *Aeluropus* were considered. Samples were obtained from specimens at Alzahra University Herbarium (ALUH) and Herbarium of Iranian Research Institute of Plant Protection, Department of Botany (IRAN). They were identified using taxonomic literatures such as Flora Iranica (Bor 1970), Flora of Iraq (Bor 1968) and Flora of Turkey (Davis 1985). Voucher specimens and localities are mentioned in Table 1.

For lemma and palea studies, mature florets of spikelets, were chosen and complete lemmas and paleas were separated. Middle part of abaxial surfaces were examined without any treatment. At first, each part was examined by Olympus stereomicroscope and Dino Lite digital microscope. Then samples were mounted on metallic stubs, coated with gold in a sputter coater with 100 Å layer of gold and examined and photographed with Hitachi SU3500 scanning electron microscope (SEM). Eight diagnostic characters of lemma and palea were evaluated (Tables 2 and 3). Terminology was adapted from Snow (1996), Acedo and Llamas (2001), Mejía-Saules and Bisby (2003) and Liu et al. (2010) for lemma and palea characters. Barthlott et al. (1998) terminology was used for epicuticular wax variation.

Table 2. Micro-morphological characters of lemma surfaces in populations/taxa studied.

Species	Pop. no.	Characters							
		Micro-prickle	Macro-hair	Long cell out line	Cork cell	Silica cell	Papillae	Bicellular micro-hair	Epicuticular wax
A. littoralis	1	a-type	absent	wide U-shaped	absent	dumbbell-shaped	on long cell	long base-cell	granule, platelet
	2	a-type, b-type	papilla-base	wide U-shaped	oblong	dumbbell-shaped	on long and short cells	long base-cell	platelet, granule
	3	absent	absent	straight	oblong	cross-shaped	on long and short cells	long base-cell	platelet, granule
	4	a-type	absent	straight	absent	dumbbell-shaped	on long and short cells	short base-cell	granule, platelet
A. lagopoides	5	absent	geniculate, papilla-base	wide U-shaped	absent	dumbbell-shaped	on long cell	long and short base-cell	granule
	6	a-type, b-type	absent	wide U-shaped	squared	dumbbell-shaped	on long and short cells	long and short base-cell	cube, granule
	7	absent	geniculate	straight	absent	dumbbell-shaped	on long and short cells	long and short base-cell	granule, platelet
	8	absent	papilla-base	wide U-shaped	absent	dumbbell-shaped	on long and short cells	long base-cell	platelet
A. macrostachyus	9	absent	geniculate	Ω-shaped	oblong	dumbbell-shaped	on long and short cells	long base-cell	granule
	10	a-type	geniculate	Ω-shaped	absent	dumbbell-shaped	on long and short cells	long base-cell	granule, platelet

Table 3. Micro-morphological characters of palea surfaces in populations/taxa studied.

Species	**Pop. no.**	**Characters**							
		Micro-prickle	Macro-hair	Long cell out line	Cork cell	Silica cell	Papillae	Bicellular micro-hair	Epicuticular wax
A. littoralis	1	b-type	absent	Ω-shaped	absent	cross- shaped	on long cell	absent	cube, granule
	2	a-type, b-type	geniculate	Ω-shaped	absent	cross- shaped	absent	absent	platelet, granule
	3	a-type, b-type	absent	Ω-shaped	oblong	absent	absent	short base-cell	granule, platelet
	4	absent	absent	Ω-shaped	crescent-shaped	cross- shaped	on long cell	absent	platelet, granule
A. lagopoides	5	absent	absent	wide U-shaped	crescent-shaped	absent	absent	absent	granule
	6	a-type	absent	Ω-shaped	scalariform, oblong	absent	on long cell	short base-cell	granule
	7	absent	absent	wide U-shaped	oblong	absent	absent	absent	platelet, granule
	8	c- type	absent	wide U-shaped	oblong, crescent-shaped	absent	absent	absent	platelet
A. macrostachyus	9	b-type, c-type	absent	Ω-shaped	absent	saddle-shaped	absent	absent	cube, granule
	10	a-type	absent	Ω-shaped	absent	cross- shaped	on long cell	absent	granule

Results

Lemma micromorphology

Lemma micromorphological details of each population are summarized in Table 2. Outline of long cell in intercostal areas were straight to wide U-shaped in *A. littoralis* and *A. lagopoides* and Ω-shaped in *A. macrostachyus* (Figs. 1-3). Short cells were found in species studied. Cork cells were absent in most populations studied. Cork cells were only observed in Bar-Ahuyi and Talkheh Rud population of *A. littoralis* (Figs. 1B-C). Zeh-e-kalut population of *A. lagopoides* showed square cork cells (Fig. 2b). Three other populations did not have cork cells.

In *A. macrostachyus*, Kahnooj population had small oblong cork cell. They were frequent in costal zones with dense distribution (Fig. 3a).

Dumbbell-shaped silica cells were found in all taxa. In Talkheh Rud population of *A. littoralis*, cross-shaped silica cells were determined. This population had the densest distribution of silica cells among other populations of *A. littoralis* (Fig. 1c). The density of silica cells in *A. macrostachyus* was the highest among species studied (Fig. 3).

Papillae occurred on long and short cells of lemma surface in populations/taxa studied. They were only observed on long cells in Kahnooj population of *A. littoralis* (Fig. 1A) and Garmsar population of *A. lagopoides* (Fig. 2A).

Macro-hairs showed variation in length and density. These unicellular structures were densely covered the lemma surface of *A. lagopoides* (especially in Garmsar population) (Fig. 2A) while in *A. macrostachyus* they were sparse. In *A. macrostachyus*, geniculate macro-hairs were seen (Fig. 3) while in *A. lagopoides*, two types of macro-hairs, geniculate and papilla- base, were seen (Fig. 2). Except Bar-Ahuyi population, there was no macro-hair in *A. littoralis* populations (Fig. 1).

In lemma and palea, three types of micro-prickles were observed: barbs developed from the apex of the base with direct point (a- type), barbs with direct point not developed from the base (b-type) and barbs with recurved point not developed from the apex of the base (c-type) (adapted from Ellis 1979). Micro-prickles were commonly found in *A. littoralis* lemma surface (Fig. 1) but in two other taxa, these features were sparse.

Salt glands were observed in all taxa studied. These bicellular excretory organs were commonly found in coastal areas of lemma epidermis. Taxa studied showed chloridoid type of bicellular micro-hairs. This type of micro-hair was classified to two sub-types: short-base cell and long-cell base. *A. macrostachyus* had long-cell base type (Fig. 3) but *A. littoralis* and *A. lagopoides* had both types (Figs. 1-2).

Epicuticular wax showed differences in type and distribution among taxa/population studied. In *A. littoralis*, platelet and granule waxes were seen in populations. These waxes were densely covered the lemma surface of Saveh population (Fig. 1D-d). Among populations of *A. lagopoides*, Garmsar population showed the densest cover of granular wax (Fig. 2a). Zeh-e-kalut population showed sparse distribution of cube and granule wax. Other populations showed medium coverage of platelet and granule

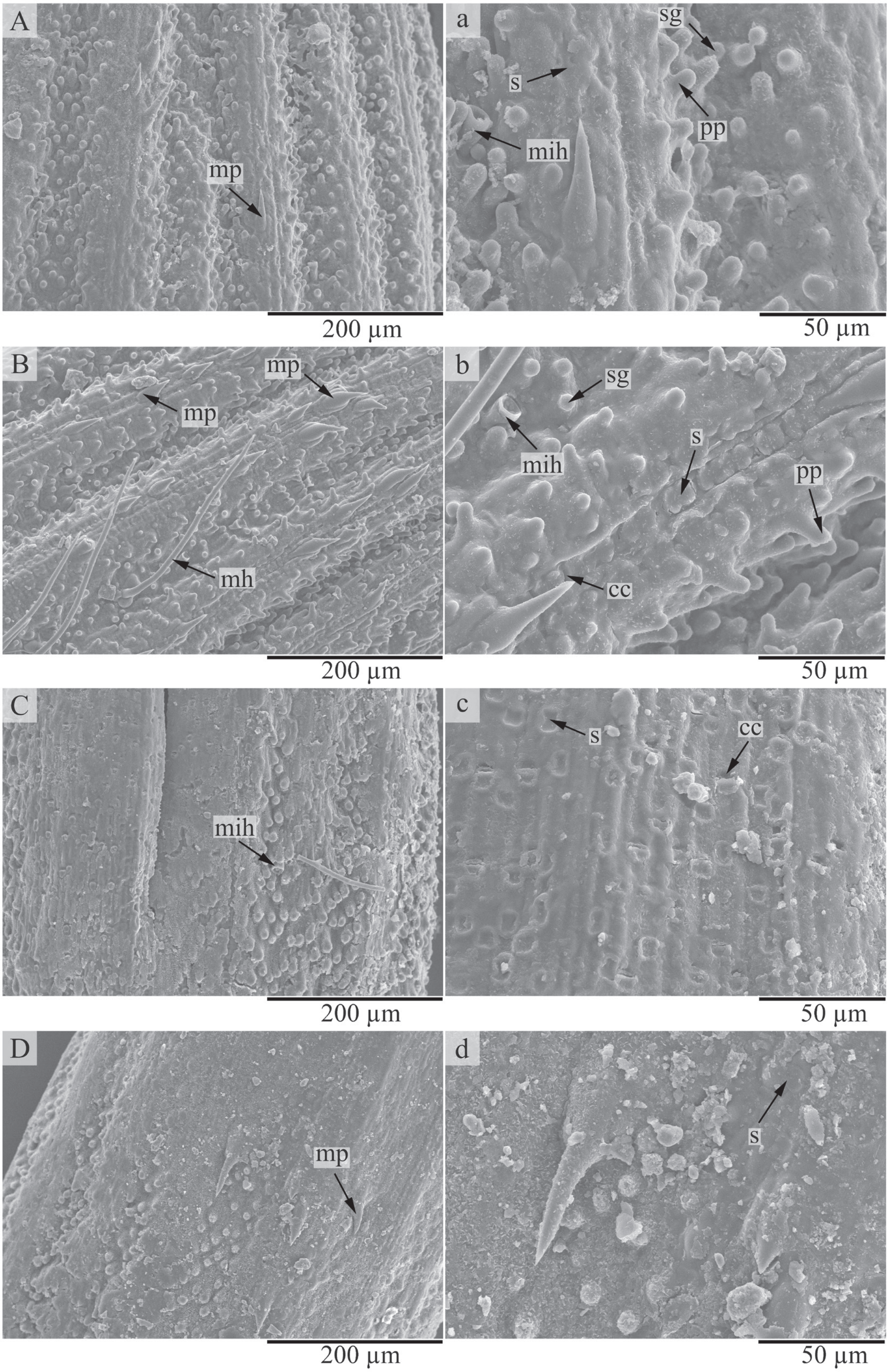

Figure 1. SEM micrographs of lemma surfaces in *A. littoralis*. A-a: Kahnooj; B-b: Bar-Ahuyi; C-c: Talkheh Rud; D-d: Saveh. cc: cork cell; s: silica cell; mh: macro-hair; mih: micro-hair; mp: micro-pickle; pp: papillae; sg: salt gland.

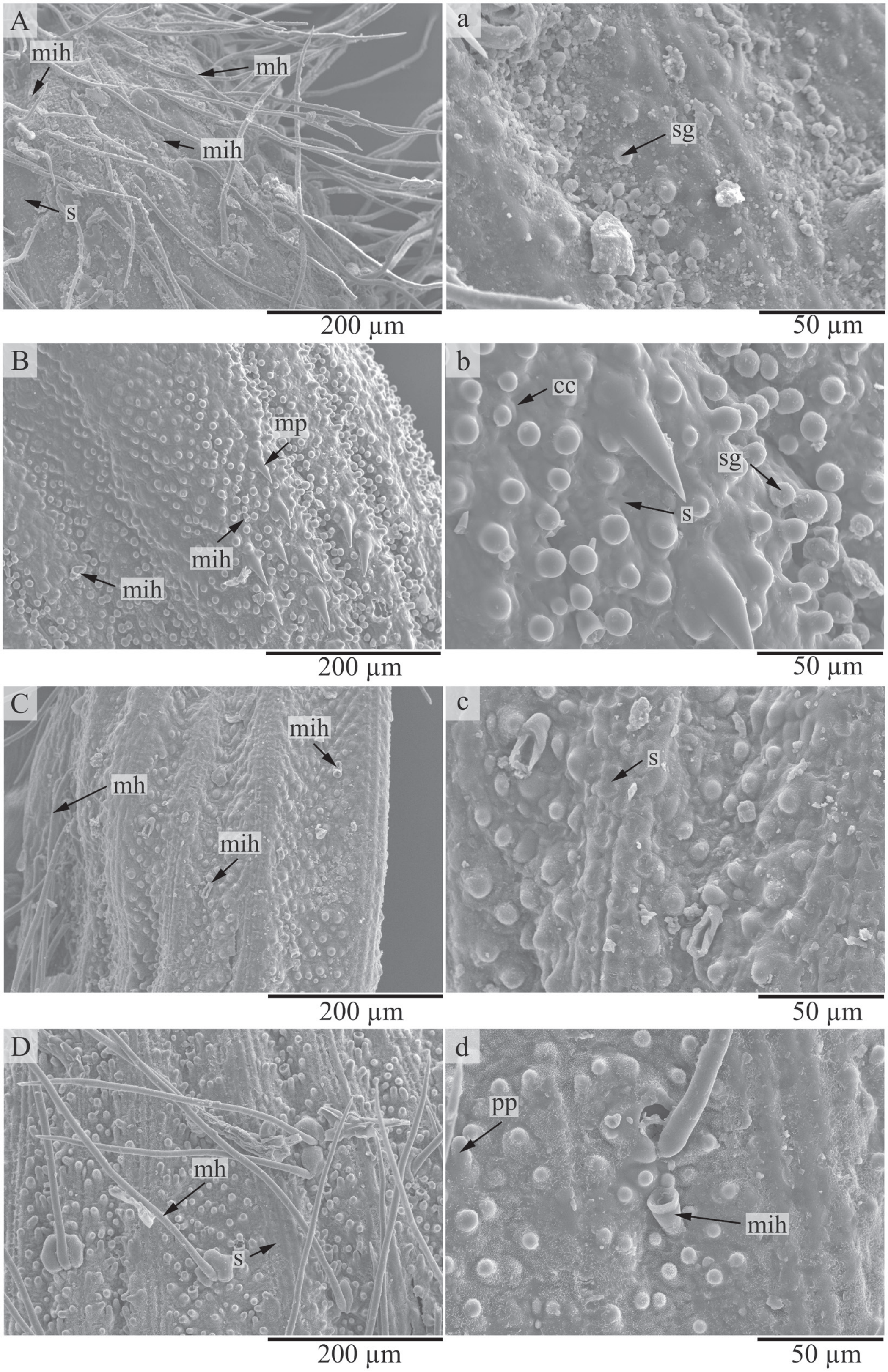

Figure 2. SEM micrographs of lemma surfaces in *A. lagopoides.* A-a: Garmsar; B-b: Zeh-e-kalut; C-c: Bar-Ahuyi; D-d: Maharlu lake. cc: cork cell; s: silica cell; mh: macro-hair; mih: micro-hair; mp: micro-pickle; pp: papillae; sg: salt gland.

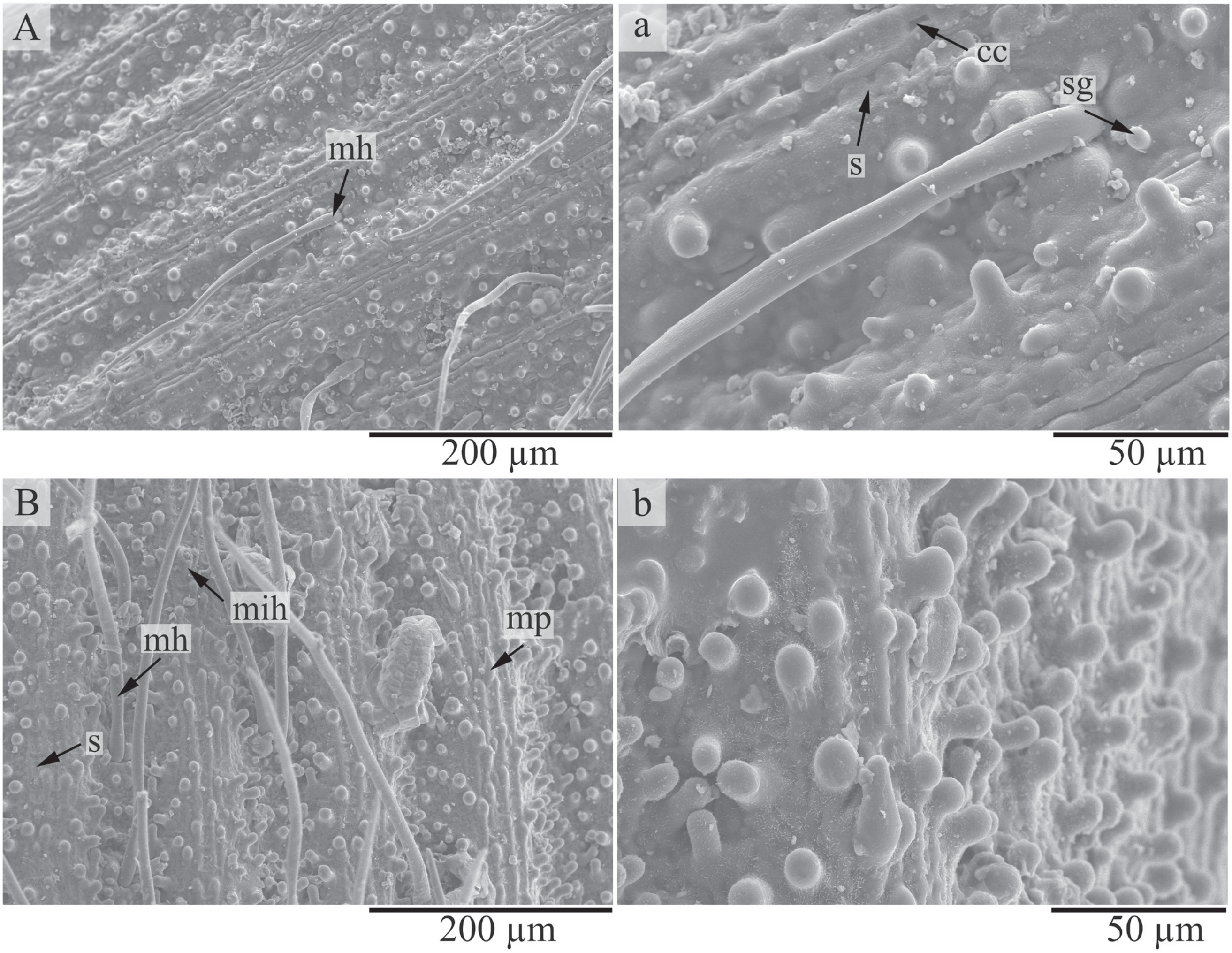

Figure 3. SEM micrographs of lemma surfaces in *A. macrostachyus*. A-a: Kahnooj; B-b: Mirjaveh to Jaleq. cc: cork cell; s: silica cell; mh: macro-hair; mih: micro-hair; mp: micro-pickle; sg: salt gland.

wax types (Fig. 2). Lemma surface of *A. macrostachyus* was sparsely covered with granule and platelet waxes (Fig. 3).

Palea micromorphology

A. littoralis and *A. macrostachyus* showed Ω-shaped outline in long cells (Figs. 4, 5E-F) while *A. lagopoides* showed wide U-shaped in long cells outline except Zeh-e-kalut population (Fig. 5A-D) (Table 3). *A. littoralis* and *A. lagopoides* showed cork cells in some populations. *A. littoralis* had oblong and crescent- shaped cork cells (Fig. 4D) but populations of *A. lagopoides* showed modifications in shape of cork cells (Fig. 5A-D). Cork cells were not observed in *A. macrostachyus* (Fig. 5E-F). Silica cells were only observed in *A. littoralis* and *A. macrostachyus*. Two populations of *A. macrostachyus* had saddle- shaped and cross- shaped silica cells (Fig. 5E-F) while in *A. littoralis* only cross-shaped ones were found (Fig. 4). Bar-Ahuyi population of *A. littoralis* had the densest distribution of silica cells and Kahnooj population of *A. macrostachyus* had the sparest.

Papillae were found on long cells of four populations studied. Macro-hairs were lacking in species studied except Bar-Ahuyi population of *A. littoralis* (not shown). In *A. littoralis*, all populations had micro-prickles except Saveh population. In *A. lagopoides*, only Maharlu lake and Zeh-e-kalut populations had micro-prickles. Bar-Ahuyi population of *A. littoralis* and Maharlu lake population of *A. lagopoides* showed more micro-prickles among other populations mostly distributed in the margin (not shown).

Chloridoid type of bicellular micro-hairs were only present in Talkheh Rud population of *A. littoralis* (Fig. 4C) and Zeh-e-kalut population of *A. lagopoides* (not shown). These two populations showed short-base cell chloridoid type.

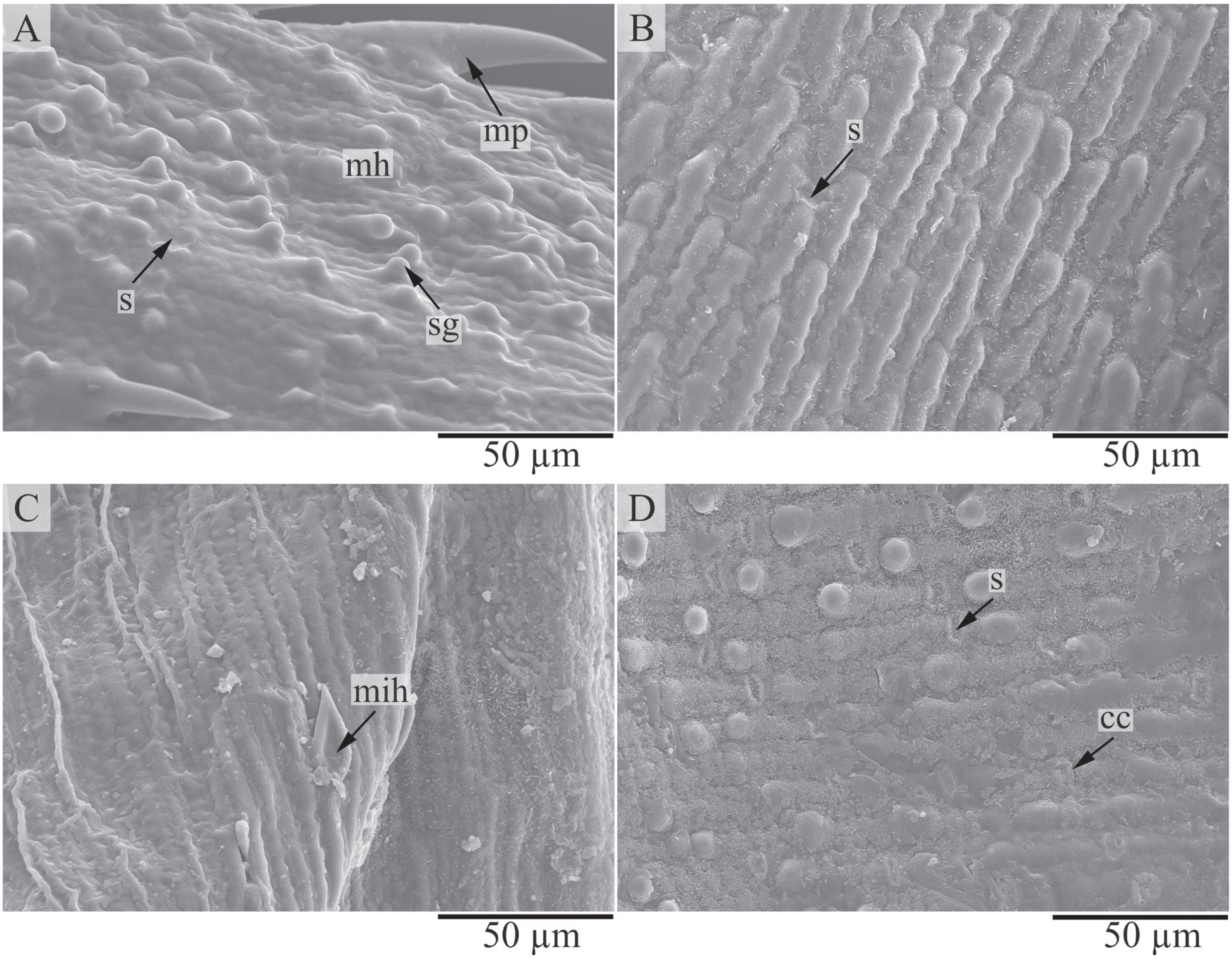

Figure 4. SEM micrographs of palea surfaces in *A. littoralis*. A: Kahnooj, B: Bar-Ahuyi, C: Talkheh Rud, D: Saveh. cc: cork cell, s: silica cell, mih: micro-hair; mp: micro-pickle, sg: salt gland.

Dense platelet waxes covered palea surface of Saveh population in *A. littoralis*. A little number of granules were also seen in this population (Fig. 4D). Platelet and granular waxes were irregularly distributed in palea surfaces of Bar-Ahyui and Talkheh Rud populations (Fig. 4B-C). Wax was scarce in Kahnooj population. In populations of *A. lagopoides*, granule and platelet waxes were seen with dense distribution except Zeh-e-kalut population (Fig. 5A-D). In *A. macrostachyus* wax was rare (Fig. 5E-F).

Discussion

Aeluropus is a halophyte plant distributed in dry and saline regions of different parts of Iran (Bor 1970). This grass showed dumbbell-shaped, cross-shaped and saddle-shaped silica cells in lemma and palea surfaces of nearly all populations studied. These features enable *Aeluropus* to live in water deficiency (Santi et al. 2018). In addition, Chloridoid type of salt glands in the form of long base-cells and short base-cells microhairs were scattered in the different parts of *Aeluropus* accessions that enable them to tolerate high salinity (Kobayashi 2008; Céccoli et al. 2015). We found epicuticular wax in shapes of cube, granule, and platelet with different density on the lemma and palea surfaces showing adaptation in different conditions.

The natural occurrence of this plant in saline habitats makes it a useful candidate for stabilizing the soil. Moreover, this plant can be used as forage especially in salty and dry soils where little fodders can grow (Zhang et al. 2006; Barzegargolchini et al. 2017).

In this study, the highest level of silica body occurred in lemma surface of *A. macrostachyus*. Presence of more silica cells cause less grazing (Quigley and Anderson

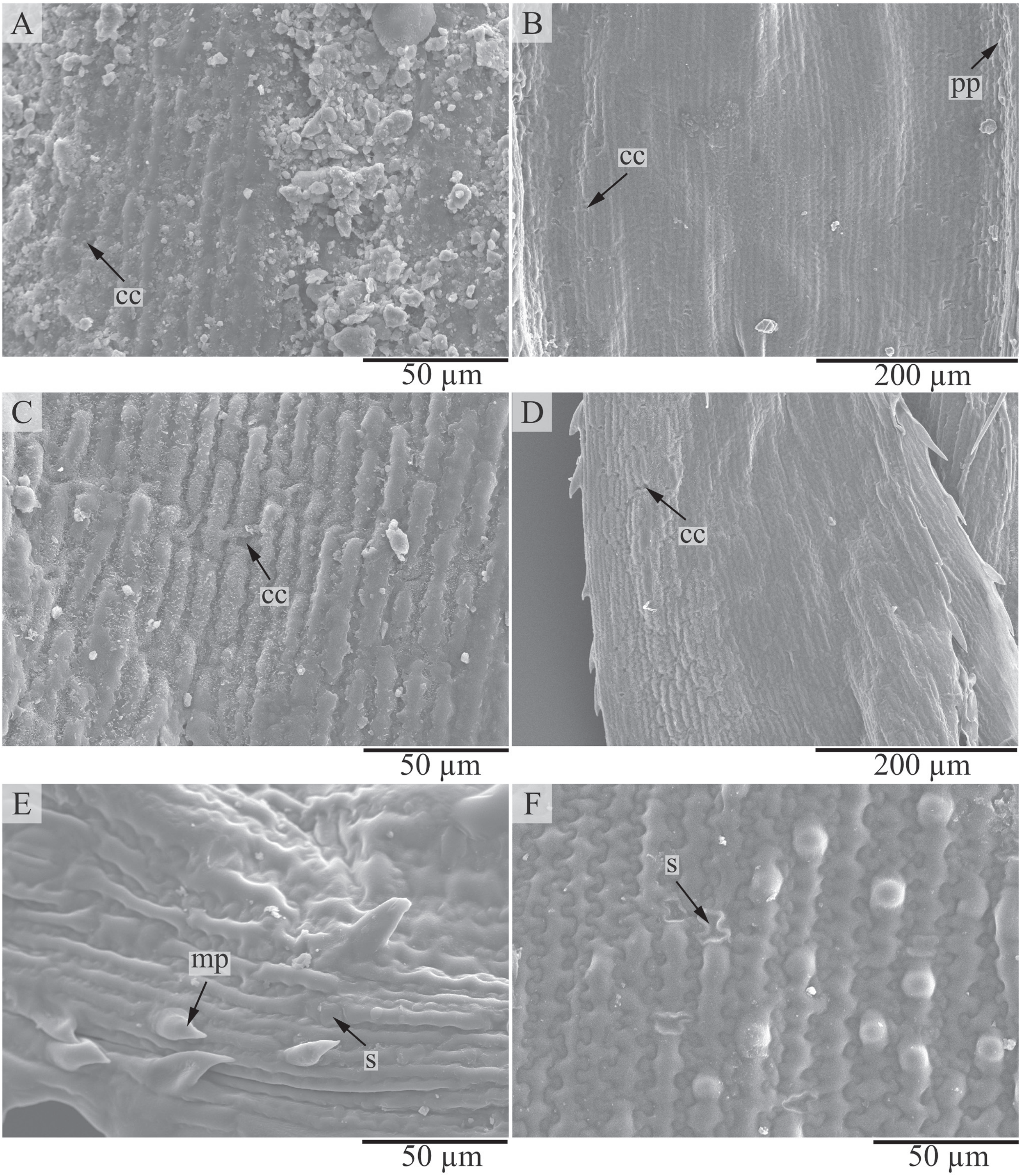

Figure 5. SEM micrographs of palea surfaces in *A. lagopoides*. A: Garmsar, B: Zeh-e-kalut, C: Bar-Ahuyi, D: Maharlu lake; E-F: *A. macrostachyus*; E: Kahnooj; F: Mirjaveh to Jaleq. cc: cork cell; s: silica cell; mp: micro-pickle; pp: papillae.

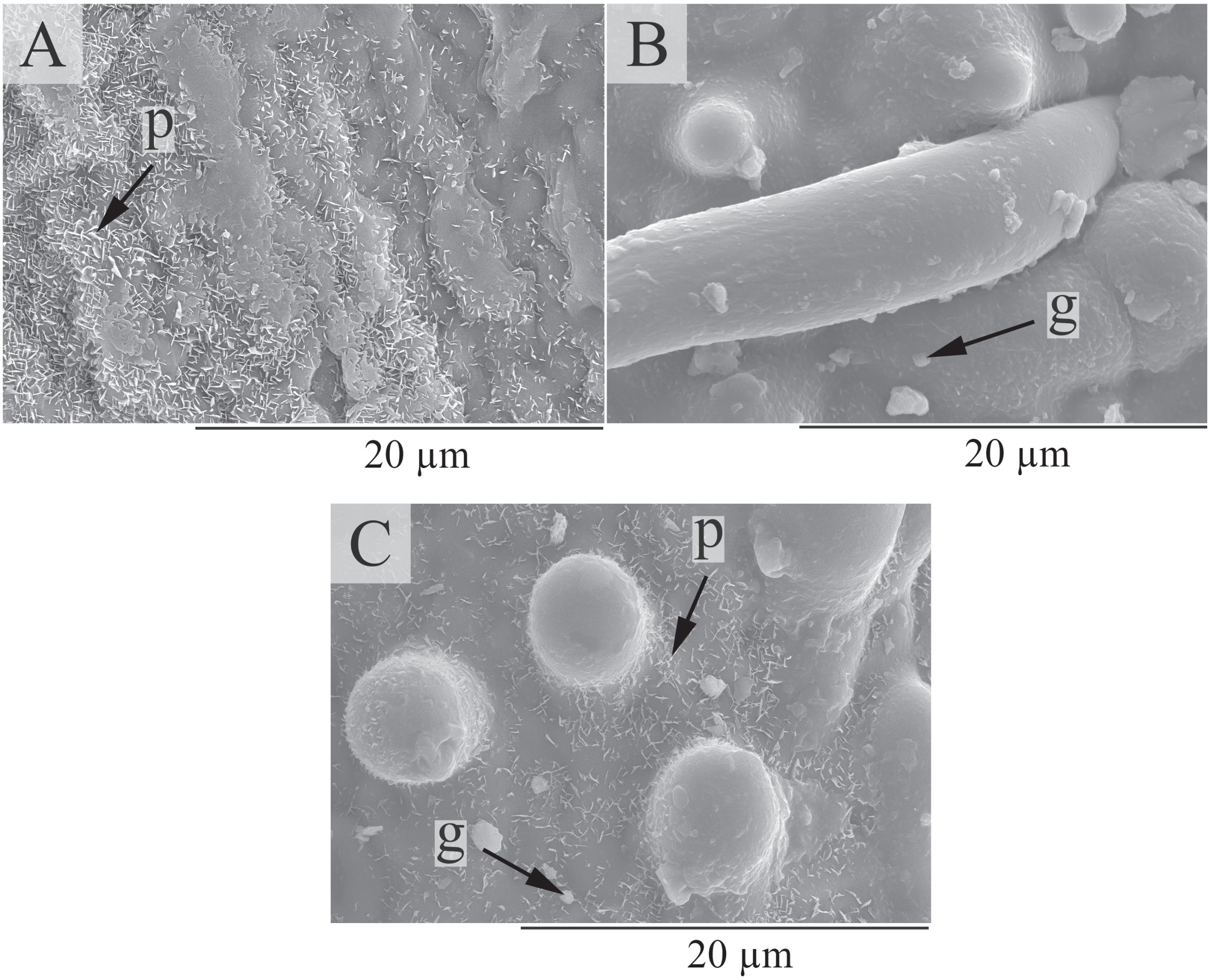

Figure 6. SEM micrographs of lemma and palea surfaces. A: platelet waxes in lemma surface of *A. lagopoides* (Maharlu lake population); B: granule waxes in lemma surface of *A. macrostachyus* (Mirjaveh to Jaleq population); C: platelet and granule waxes in palea surface of *A. littoralis* (Saveh population). p: platelet wax; g: granule wax.

2014). It maybe because of this, foraging quality of two palatable taxa, *A. littoralis* and *A. lagopoides* were studied more by different authors (Abbasi et al. 2002; Sharifi Rad et al. 2013).

All species possessed papillae, silica cells and salt glands on lemma surfaces. Except four populations (no. 2, 3, 6, 9), we did not find cork cells on lemma surfaces. Despite palea, papilla-base and geniculate macro-hairs occurred in lemma surface of most populations.

The results of our study suggest that lemma and palea micromorphology may be useful to distinguish *Aeluropus* species. Although some characters as shape of silica cells in lemma and absence of macro-hairs in palea were uniform among taxa studied, some others differed among species. For example, *A. macrostachyus* was the only species with Ω-shaped outline in long cells of lemma and with no salt gland on palea surface. Our results confirmed its distinct position based on morphological characters as Abivardi et al. (2010) mentioned before. Liu et al. (2010) noted to U-shaped outline for long cells and dumbbell shape for silica cells in lemma of *A. littoralis*. Our results were in agreement with them. Despite some overlapping features, generally our micro-morphological results separate the three species studied.

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