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A new *Aculodes* species (Prostigmata: Eriophyoidea: Eriophyidae) associated with medusahead, *Taeniatherum caput-medusae* (L.) Nevski (Poaceae)

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Abstract

A new species of plant mite (Acari: Eriophyidae) was discovered on medusahead (*Taeniatherum caput-medusae*), an annual grass that is native to central Asia and the Mediterranean Basin. It is invasive in western North America. *Aculodes altamurgiensis* **sp. nov.**, is described here and differentiated from other *Aculodes* spp., on the basis of morphology. Its DNA fingerprinting was reported and compared with *Aculodes mckenziei* collected from *Elymus repens* and *Bromus inermis*. Pairwise comparison of MT-CO1 sequences between *A. altamurgiensis* **sp. nov.,** and *A. mckenziei* revealed 20.2–21.5% genetic divergence between these congeneric species. First collected in Parco Nazionale dell'Alta Murgia in Apulia, Italy in 2014, *A. altamurgiensis* **sp. nov.**, has been subsequently collected from medusahead in Serbia, Bulgaria, Iran and Turkey. Based on these data and on preliminary observations on the effects of the mite on plant growth, *A. altamurgiensis* **sp. nov.,** is currently being investigated as a candidate biological control agent of medusahead.

Key words: Apulia, biological control, conservation, grasses, weeds

Introduction

All mites in the family Eriophyidae are obligate herbivores. Many of them are crop pests, some of which can transmit viruses (Petanović & Kielkiewicz 2010); while others are associated with weeds and have high potential as classical biological control agents (Rosenthal 1996; Smith *et al*. 2010). Several eriophyid species have been released as biological control agents but to date, none have been released to control grass targets (Smith *et al.* 2010; Winston *et al*. 2014).

Species in the genus *Aculodes* Keifer associated with grass hosts are found in many geographic regions; most of them have been reported from the northern hemisphere (de Lillo & Amrine, unpublished database). To date, about 30 species have been described in the genus *Aculodes*, of which most were recorded from grasses (Poaceae). The others were found on hosts in the families Malvaceae, Salicaceae, Leguminosae and Rosaceae (Alemandri *et al*. 2015, Boczek & Chandrapatya 1998; Huang 1992, 2001; Keifer 1944, 1952, 1960, 1966a, 1966b; Kuang 1997; Kuang & Pang 1997; Kuang *et al*. 2005; Nalepa 1891; Shi & Boczek 2000; Skoracka 2003, 2004, 2005; Skoracka & Pacyna 2005; Skoracka *et al*. 2001, 2009; Sukhareva 1972, 1981, 1985, 1986, 1994; Xue *et al*. 2010, 2012).

Medusahead, *Taeniatherum caput-medusae* (L.) Nevski (Poaceae), is a winter annual grass native to western Asia and the Mediterranean Basin (Frederiksen 1986). It has become invasive in western North America, spreading rapidly and degrading rangeland habitats (Young & Evans 1970; Davies & Johnson 2008). It is currently the target of a classical biological control program, with a focus on the discovery and development of eriophyid mite natural enemies as biological control agents (Rector B.G., pers. comm.). In the course of native range surveys for eriophyid mites on medusahead, an unidentified *Aculodes* sp., was collected in the Parco Nazionale dell'Alta Murgia in Apulia, Italy, as well as in Serbia, central Turkey, northern Iran and Bulgaria. This report presents the description of this new eriophyoid mite species, *Aculodes altamurgiensis* **sp. nov.**

Material and methods

Collection and Light Microscopy Morphological Study

Plant samples of *T. caput-medusae* were collected from Italy, Serbia, Bulgaria, Turkey, and Iran from 2014 to 2017 and examined in the laboratory. Mites were removed from the plants using a fine needle under a dissection stereomicroscope and by using extraction methods described by de Lillo (2001) and Monfreda *et al*. (2007). Mites were mounted in Keifer's F medium (Amrine & Manson 1996) and then examined using two phase-contrast microscopes (Leica DMLS, Wetzlar, Germany; Olympus BX50, Hamburg, Germany). Morphology and nomenclature follow Lindquist (1996) and genus classification is based on Amrine *et al*. (2003). Measurements and illustrations were made according to Amrine and Manson (1996) and de Lillo *et al*. (2010). Morphometry was performed using the software package IM 1000 (Leica, Wetzlar, Germany) and for hand-drawn line art a camera lucida was used. All measurements are given in micrometers (μm) and, unless stated otherwise, are the length of the structure. Plant names are in accordance with The Plant List (2013) on-line database.

The holotype and the paratype slides are deposited in the collections of the Acarology Laboratory, Department of Entomology and Agricultural Zoology, Faculty of Agriculture, University of Belgrade, Serbia; one paratype slide is deposited at the Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti (DiSSPA), University of Bari Aldo Moro, Italy.

Scanning Electron Microscopy Study

Scanning electron micrographs (SEM) were taken according to Nuzzaci and Vovlas (1976) (see also Alberti & Nuzzaci 1996). Live mites were collected individually using a fine pin from fresh plant material under a stereomicroscope. For specimen preparation before being placed on the SEM stage, mites were sputter-coated with gold for 100 s under 30 mA ion current. The mites were then studied in the vacuum chamber of a JEOL Scanning Electron Microscope (JEOL-JSM6390, Peabody, MA, USA) at the Laboratory of Electron Microscopy, Faculty of Agriculture, University of Belgrade, Serbia.

DNA Extraction, PCR Amplification and Sequencing

Material collected for molecular analysis included populations from Italy and Serbia (Table 1). Mites for DNA extraction were collected from fresh plant material, preserved in 96% ethanol and stored at -20 °C. Total genomic DNA was extracted from a pool of 20 whole mites using a QIAGEN DNeasy® Blood & Tissue Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. DNA samples are archived at -80 °C at the Institute for Plant Protection and Environment (Belgrade, Serbia).

Amplification of a 658 bp barcode fragment of subunit I of the mitochondrial cytochrome c oxidase gene (MT-CO1) was performed in 25 µl, using primers LCO1490 and HCO2198 (Folmer *et al*. 1994), following reaction conditions specified by Chetverikov *et al*. (2012). PCR products were separated by electrophoresis through a 1% agarose gel in TBE buffer (Tris-Borate 90 mM, EDTA 1 mM), then stained with ethidium bromide for visualization under a UV transilluminator.

Amplicons were purified using the QIAquick PCR purification Kit (QIAGEN) according to the manufacturer's instructions and then sequenced on automated equipment by Macrogen (Seoul, South Korea) with the HCO2198 primer (the reverse primer in the initial PCR procedure). The sequences were manually edited using FinchTV v. 1.4.0 (www.geospiza.com), and aligned by CLUSTAL W (integrated within MEGA5 software; Tamura *et al*. 2011). Uncorrected pairwise genetic distances were used to calculate the average genetic distance between *A. altamurgiensis* **sp. nov.,** populations associated with *T. caput-medusae* collected from Italy and Serbia (Table 1).

TABLE 1. Collection data for *Aculodes altamurgiensis* **sp.** nov., inhabiting *Taeniatherum caput-medusae* populations.

	Country	Locality,—date	GPS coordinates	Name of collector
1	Italy	Castel del Monte, May 2015	41˚03'58.2"N, 16˚15'21.6"E	Cristofaro M.
2	Serbia	Krševica, May 2016	42˚25'51"N, 21˚52'5"E	Rector B.
3	Serbia	Aleksandrovačko jezero, May 2016	42˚29'11"N, 21˚53'48"E	Rector B.

Sequences of *A. altamurgiensis* **sp. nov.,** were trimmed and compared with the two corresponding sequences of *Aculodes mckenziei* (Keifer), collected from *Elymus repens* and *Bromus inermis,* which are available in the NCBI database (GenBank accession numbers FJ387561 and FJ387562). Currently, these *A. mckenziei* sequences are the only COI sequences of any *Aculodes* spp., that have been recorded in GenBank.

Results

Aculodes altamurgiensis sp. nov., de Lillo & Vidović

Description. FEMALE (n=10). Body wormlike 225 (212–286), 53 (53–67) wide, whitish in color. **Gnathosoma** 19 (19–20) curved downwards, chelicerae 14 (14–16), setae *ep* 3, setae *d* 7 (7–9) unbranched. **Prodorsal shield** 36 (32–42) including the frontal lobe, 29 (28–33) wide, triangular with a pronounced, elongated and pointed frontal lobe over the gnathosoma; median line present in the posterior half of the shield; admedian lines complete, subparallel; I pair of submedian lines present, paired, incomplete, subparallel to admedian, in the central part of the shield; II pair of submedian lines present, paired, incomplete, parallel to lateral shield margins; dashes present on rear surface of the shield and between the lines. Tubercles *sc* subcylindrical, on rear shield margin 24

(22–25) apart, scapular setae *sc* 39 (39–53). **Leg I** 34 (33–36); femur 9 (8-10), setae *bv* 18 (13–19); genu 6 (6–7), setae *l″* 25 (24–29); tibia 8 (7–8), setae *l′* 12 (10–12); tarsus 7 (7–9), setae *ft′* 18 (18–26), setae *ft″* 7 (7–14); tarsal solenidion *ω* 10 (8–10) with a thinner and rounded end; tarsal empodium 8 (6–9), 7-rayed. **Leg II** 33 (31–34); femur 9 (9-10), setae *bv* 17 (14–21); genu 6 (5–6), setae *l″* 17 (16–25); tibia 7 (6–7); tarsus 8 (7–8), setae *ft′* 26 (20–28), setae *ft″* 10 (9–12); tarsal solenidion *ω* 9 (8–11) similar to that on leg I; tarsal empodium 10 (7–10), 7-rayed. **Coxae** sparsely granulated and with short lines; sternal line 9 (9–11); setae *1b* 9 (9–11), tubercles *1b* 9 (9–13) apart; setae *1a* 15 (13–24), tubercles *1a* 8 (8–13) apart, setae *2a* 28 (23–44), tubercles *2a* 23 (19–27) apart. **Genital coverflap** 11 (11–15), 21 (18–22) wide, with 10 (10–11) longitudinal striae in a single row; setae *3a* 20 (18–23), 16 (14–20) apart. **Internal genitalia** with anterior apodeme trapezoidal, longitudinal bridge relatively long, the post spermathecal part of the longitudinal bridge is reduced; spermathecal tubes directed latero-posterad, composed of two parts: basal part egg-shaped, distal part more tubulose; spermathecae globose. **Opisthosoma** with subequal annuli: 53 (51–58) dorsal and 56 (55–66) ventral annuli; 5 (5–6) coxigenital annuli. Dorsal and ventral opisthosoma with pointed microtubercles close to the rear margins of annuli. Setae *c2* 35 (30–43), 48 (48–53) apart, on annulus 8 (7–9); setae *d* 30 (28–41), 26 (26–40) apart, on annulus 12 (12–15); setae *e* 18 (17–31), 14 (14–20) apart, on annulus 26 (26–32); setae *f* 22 (22–29), 20 (20–24) apart, on annulus 49 (47–54); seta *h2* 51 (51–80), 10 (10–11) apart; setae *h1* 10 (7–10), 6 (6–7) apart.

MALE (n=2). Body wormlike, 211, 44 wide, whitish in color. **Gnathosoma** 18–20 curved down, cheliceral stylets 12. **Prodorsal shield** 31–34, 36 wide. Prodorsal shield tubercles on the rear shield margin 18–19 apart, setae *sc* 32–34, projecting posteriorly. Shield design similar to female. **Leg I** 26–28; femur 7, setae *bv* 11–12; genu 4–5, setae *l″* 21, tibia 5, tibial setae *l′* 5; tarsus 6, setae *ft′* 12, setae *ft″* 14–15; solenidion *ω* 6–8, empodium *em* 5–6, and 7-rayed. **Leg II** 23–26; femur 7, setae *bv* 15; genu 4, genual setae *l″* 12, tibia 5; tarsus 6; setae *ft′* 6, setae *ft″* 14–16; solenidion *ω* 8–9, empodium *em* 6. **Coxae** granulated; sternal line 6–7; setae *1b* 6–7, 1b tubercles 8 apart; setae *1a* 18, 1a tubercles 5 apart; setae *2a* 26–28, 2a tubercles 18–19 apart. **Genitalia** 19–20 wide; setae *3a* 11–16, *3a* tubercles 11–15 apart. **Opisthosoma** with subequal annuli: 57–63 dorsal and 61–63 ventral annuli; 6 coxigenital annuli. Setae *c2* 24–27, 44 apart, on annulus 9–11; setae *d* 36–37, 28–29 apart, on annulus 19–21; setae *e* 16–17, 11–13 apart, on annulus 34–35; setae *f* 14, 18 apart, on annulus 57–59; setae *h2* 55–72, 7 apart, setae *h1* 4–5, 4 apart.

Type host plant. *Taeniatherum caput-medusae* (L.) Nevski (Poaceae), medusahead.

Type locality. Road SP 234 Km 19, about 2 km west of Castel del Monte (Andria) (41.0662˚N, 16.256˚E), 504 m above sea level, Apulia, Italy

Type material. Female holotype (slide #654/3) and paratypes: 12 females, 4 males; 13 May 2015, by M. Cristofaro, A. Paolini and D. De Simone.

Additional studied materials. Castel del Monte, Apulia, Italy (41.0662˚N, 16.256˚E), 27 May 2014, 10 slides; 3 May 2016, 2 slides 8 December 2016, 11 slides; 8 February 2017, 1 slide. Davidovac (Kladovo), Serbia (44.6350˚ N, 22.5477˚ E), 1 June 2015; 15 slides; 30 May 2016, 2 slides. Krševica (Vranje), Serbia (42.4312 ˚N, 21.868 ˚E), 3 June 2015, 12 slides; 25 April 2016, 4 slides; 29 May 2016, 2 slides. Aleksandrovačko jezero (Vranje), Serbia (42.4865 ˚N, 21.8965 ˚E), 29 May 2016, 20 slides. Chernoochene, Bulgaria (41.7768 ˚N, 25.342 ˚E), 5 June 2015, 9 slides; Varovnik (Sredetz), Bulgaria (42.2191 ˚N, 27.154 ˚E), 6 June 2015, 5 slides; Barzitsa (Provadia), Bulgaria (43.0729 ˚N, 27.526 ˚E), 7 June 2015, 5 slides; Cavusin, Turkey (38.7406˚N, 35.027 ˚E), 16 April 2015, 6 slides; Soufian (East Azerbaijan), Iran (38.3178 °N, 45.943 °E), June 2017, 15 slides. Specimens collected by M. Cristofaro, F. Di Cristina, E. de Lillo, P. Lotfollahi, F. Marini, M. Augé, S. Marinković, A. Paolini, R. Petanović, B. Rector, D. Smiljanić, and B. Vidović.

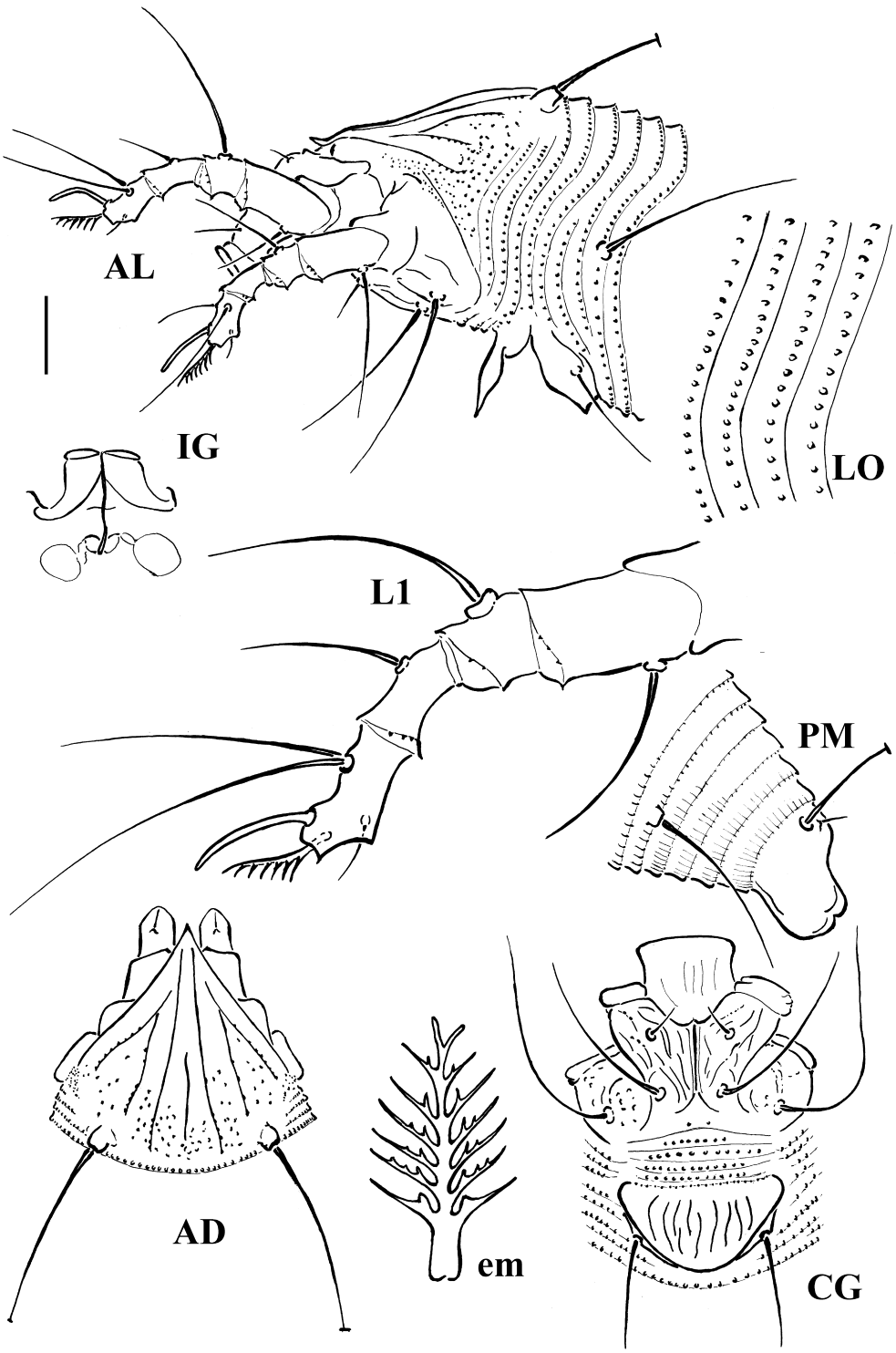

FIGURE 1. Line drawings of *Aculodes altamurgiensis* **sp. nov.**: **AD**. Prodorsal shield; **AL**. Lateral view of anterior body region; **CG**. Female coxigenital region; **em**. Empodium; **IG**. Internal female genitalia; **LO**. Lateral view of annuli; **L1**. Leg I; **PM**. Lateral view of posterior opisthosoma. Scale bar: 10 μm for **AD**, **AL**, **CG**, **GM**, **IG**, **PM**; 5 μm for **LO**, **L1**; 2.5 μm for **em**.

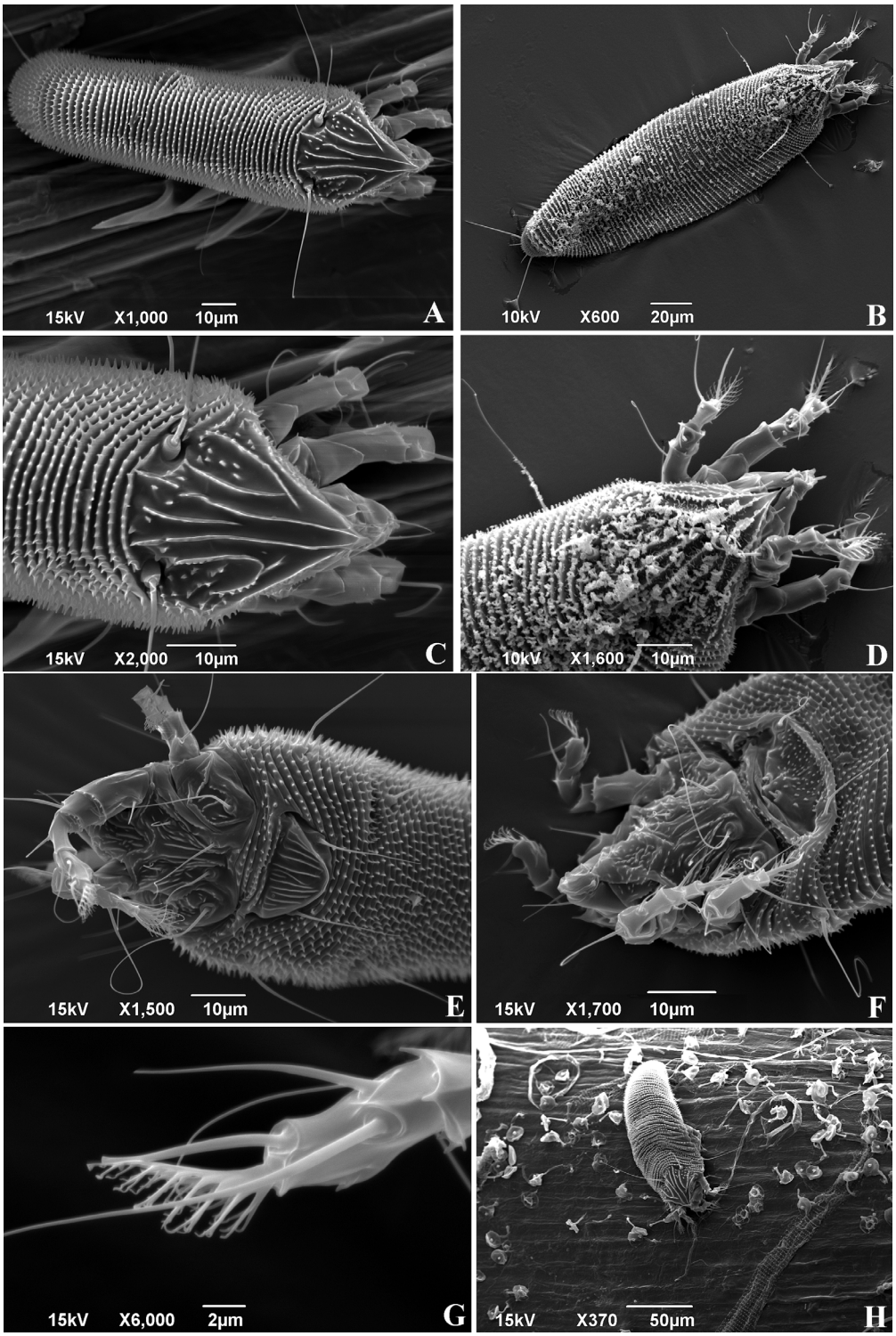

FIGURE 2. Scanning electron micrographs of *Aculodes altamurgiensis* **sp. nov.**: A. Dorsal view; B. Dorsal view with apparent wax-like secretion; C. Dorsal view with the detail of pro-dorsal shield; D. Dorsal view with detail of tarsi and apparent wax-like secretion; E. Ventral view, anterior half; F. Ventral view, anterior half; G. Detail of tarsus; H. Portion of medusahead leaf with adult and spermatophores.

Relation to the host. Mites were observed as vagrants more easily on mature medusahead plants in late spring, apparently preferring sheltered parts of the plant (e.g. on the stem protected by leaves, on ripening seeds within inflorescences). Preliminary observations indicated an impact on seed germination rate, although additional studies under controlled conditions will be necessary.

Etymology. The specific epithet *altamurgiensis* is an adjective, derived from the name of the Parco Nazionale dell'Alta Murgia, where the mite was first collected, in the plural genitive case.

Differential diagnosis and remarks. Based on the presence and length of the median line on the prodorsal shield, *A. altamurgiensis* **sp. nov.** (median line present in the posterior half of the shield) can be distinguished from species without a median line (*A. capillarisi* Skoracka, *A. dubius* (Nalepa), *A. janboczeki* Skoracka, *A. koeleriae* Sukhareva, *A. kransnovi* Sukhareva, *A. stoloniferae* Skoracka, *A. sylvatici* Skoracka, Labrzycka & Rector) and species with complete median line (*A. bambusae* Kuang, *A. levis* Huang, *A. ponticus* Sukhareva).

Aculodes altamurgiensis **sp. nov.,** can be distinguished from other *Aculodes* spp., with incomplete median lines and the same number of empodial rays based on the shape, presence, and position of their submedian lines. *Aculodes altamurgiensis* **sp. nov.,** has two submedian, incomplete,straight lines in the central part of the shield (one in the field between the tubercles *sc* and the admedian line; one in the field between the tubercle *sc* and the lateral side of the prodorsal shield) that touch each other at their anterior ends. *Aculodes calamaabditus* Skoracka and *A. deschampsiae* (Sukhareva) each have one pair of submedian lines; *A. tsukushiensis* Xue, Song & Hong has a complete pair of inner submedian lines which do not cross the outer pair of submedian lines; *A. festucae* Skoracka, Labrzycka & Rector and *A. mckenziei* (Keifer) have a pair of curved and complete outer submedian lines; *A. neglectivagrans* Skoracka has two short pairs of submedian lines that do not touch; *A. fulleri* (Keifer) has an inner pair of submedian lines is on the anterior half.

Aculodes altamurgiensis **sp. nov.,** can be distinguished from *A. mongolicus* by the length of the prodorsal shield (32–42 µm in *A. altamurgiensis* **sp. nov.** 42–46 µm in *A. mongolicus*), the width of shield (28–33 µm in *A. altamurgiensis* **sp. nov.** 42–44 µm in *A. mongolicus*), the length of *1a* setae (13–24 µm in *A. altamurgiensis* **sp. nov.** 24–33 µm in *A. mongolicus*), the length of *3a* setae (18–23 µm in *A. altamurgiensis*, **sp. nov.** 38–48 µm in *A. mongolicus*), the length of *c2* setae (30–43 µm in *A. altamurgiensis* **sp. nov.** 40–47 µm in *A. mongolicus*), the length of *d* setae (28–41µm in *A. altamurgiensis* **sp. nov.** 59–61µm in *A. mongolicus*) and length of *e* setae (17–31µm in *A. altamurgiensis* **sp. nov.** 30–38µm in *A. mongolicus*).

According to the prodorsal shield design *A. altamurgiensis* **sp. nov.,** is most similar to *Aculodes holcusi*, but there are numerous characters that differ between these two species, including number of empodial rays (*A. altamurgiensis* **sp. nov.** 7-rayed; *A. holcusi*, 8-rayed); length of *sc*, *1a*, *2a*, *c2*, *d*, *e* and *f* setae (*A. altamurgiensis* **sp. nov.,** has shorter setae in each case compared to *A. holcusi*); width of prodorsal shield (28–33 µm in *A. altamurgiensis* **sp. nov.** 49–51 µm in *A. holcusi*); length of empodium (11–15µm in *A. altamurgiensis* **sp. nov.** 17–18 µm in *A. holcusi*); number of longitudinal striae on the coverflap (10 in *A. altamurgiensis* **sp. nov.** 12 in *A. holcusi*).

Biological and ecological remarks. Surveys and field samples on native and cultivated grasses were carried out at the site near Castel del Monte, Parco Nazionale dell'Alta Murgia, where the mite was recorded for the first time on medusahead. Starting on 7 May 2015, and repeating observations after 2 weeks, twenty samples of several Poaceae (the target weed, 2 species of cultivated wheat, *Triticum durum* Desf. and *T. aestivum* L., and the wild species *Stipa capensis* Thunb., *Avena sativa* L. and *Hordeum murinum* L.) were randomly collected and inspected for the mite presence. In some of these species (*A. sativa, S. capensis*), as well on *T. caput-medusae*, eriophyid mites were recorded, but morphological identification showed that *A. altamurgiensis* **sp. nov.**, was recorded only on the target weed species (unpublished data). Adults and juveniles were collected from seedling medusahead plants in winter, indicating that females were present and reproducing. Seedling

medusahead plants infested with *A. altamurgiensis* that were collected from the field in late summer 2016 and transplanted to pots outside the laboratory (i.e. under ambient climatic conditions) grew normally and supported relatively dense populations of the mite through the summer of 2017. Closer observations under controlled conditions are required to quantify the physiological impact of *A. altamurgiensis* **sp. nov.,** on medusahead. Additional host-range resting is also required in order to assess the suitability of *A. altamurgiensis* **sp. nov.,** as a biological control agent for medusahead in the western USA.

Molecular analysis

The sequencing reaction produced a 606 bp fragment of the MT-CO1 barcode region. Base pair frequencies showed that the region is AT-rich (61.3% and 61.9% for the Italian and Serbian populations, respectively). The translation of the nucleotide sequences resulted in a 202 amino acid positions.

Two different haplotypes were obtained for populations of *A. altamurgiensis* **sp. nov.,** collected in Italy and Serbia, with no insertions or deletions occurring between the respective MT-CO1 sequences. Sequence comparison revealed minor genetic variability represented with 8 singleton polymorphisms, resulting in no amino-acid replacement. Genetic divergence over the sequence pairs was 1.3% (Table 2). The sequences are available from GenBank under accession numbers #MH352403, #MH352404, and #MH352405.

TABLE 2. Uncorrected p-distance among *Aculodes altamurgiensis* **sp. nov.,** populations collected from *Taeniatherum caput-medusae* at different localities.

	Population—Locality	1	2	3	GenBank accession no.
1	Italy—Castel del Monte				#MH352403
2	Serbia—Krsevica	0.013			#MH352404
3	Serbia—Aleksandrovacko jezero	0.013	0.00		#MH352405

Pairwise comparison of overlapping MT-CO1 fragments (531 nt) between *A. altamurgiensis* **sp. nov.,** from Italy and *A. mckenziei* collected from *Elymus repens* and *Bromus inermis* resulted in 20.2% and 21.5% genetic divergence, respectively. Populations of *A. altamurgiensis* **sp. nov.,** from Serbia showed 20.2% and 21.3% sequence divergence between the congeneric species from *Elymus repens* and *Bromus inermis*, respectively. Translation into amino acid sequences revealed 4.5% divergence between populations of *A. altamurgiensis* **sp. nov.,** from both Italy and Serbia, and *A. mckenziei* inhabiting both *Elymus repens* and *Bromus inermis.*

The morphometric results presented in this study suggest that *A. altamurgiensis* **sp. nov.,** is a distinct species. This data, combined with the observed variation between CO1 sequences of *A. altamurgiensis* and the two *A. mckenziei* samples from different hosts provide additional, persuasive evidence of this assertion.

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