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Description of the second instar larva of *Thermothrips mohelensis* Pelikán (Thysanoptera: Thripidae)

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Thermothrips mohelensis Pelikán is an extremely rare thrips found in dry steppe grassland ecosystems (Pelikán 1949, 1995; Ulitzka 2019a). It has been reported from only a few locations and almost always in very low abundance: one female each has been collected in Germany (Ulitzka 2019a), Slovenia (Kucharczyk 2008) and Turkey (Tunç 1992), two females are known from Iran (Mirab-balou & Chen 2013), three from Poland (Zawirska 1988; Kucharczyk 2007) and six from Western Russia (Schliephake 1977). Larger series including males and larvae are known only from Czechoslovakia (Pelikán 1949, 1995) and from Yakutia, East Siberia where—besides other specimens—the larvae discussed below have been collected (see Evdokarova & Kucharczyk 2020). The fragmented and selective occurrence of this species in steppe habitats has been interpreted to represent scattered relict populations that may have remained from a more extensive distribution far back, possibly in the wide dry grasslands of the Late Pleistocene (Ulitzka 2019a). Similar to other specialist species of those barren habitats *T. mohelensis* is severely threatened by the general decline of these fragile ecosystems due to natural succession or anthropogenic impact (Kucharczyk 2008; Kucharczyk & Kucharcyk 2008; Ulitzka 2019a). *T. mohelensis* is a thermophilous (zur Strassen 2003) and highly xerophilous species. This thrips was erroneously considered an hydrophilous wetland thrips (Bhatti 1998) due to a mistranslation (see Ulitzka 2019a). *T. mohelensis* lives and breeds monophagously in flowers of *Galium* (Rubiaceae), particularly *G. verum* but also *G. mollugo* (Pelikán 1949, 1995; Schliephake 1972; Tunç 1992).

The genus *Thermothrips* is attributed to the *Anaphothrips* genus-group and closely related to *Rubiothrips*. Similar to members of this genus adults of *Thermothrips* have the median setae on abdominal tergites II–IV much longer than the distance between their bases, and on tergite VIII a posteromarginal comb is lacking (Schliephake & Klimt 1979; Ulitzka 2019a, 2019b). Various character states, however, clearly differentiate *Thermothrips* from *Rubiothrips*; for example the presence of distinct pronotal posteroangular and posteromarginal setae, or the unusual combination of a simple sense cone on antennal segment III and a forked one on IV. Detailed descriptions of adults including line drawings and photomicrographs have been provided by Pelikán (1949) and Ulitzka (2019a, b). The description of larvae collected with the type specimens was announced by Pelikán (1949) but never published. The objective of the present study is to characterise the second instar larva of this rare thrips and to provide information differentiating it from similar species.

The specimens of *T. mohelensis* considered here include males, females and second instar larvae of both sexes. The samples were collected in two series at one and the same location on *Galium verum*. The first series came from one single plant by beating it onto white paper. Even though no other thrips had been recorded on this plant nor in the adjacent vegetation, we strived to exclude any risk that the larvae might have come from another *Anaphothrips* species. Therefore, a second series was collected and—after ensuring morphological conformity of the specimens of both samples—these animals were examined by ITS-RFLP analysis according to Moritz *et al.* (2002, 2004). The used primer pairs (28z/P1, 18j/O1and Cs249/Cs250) and restriction enzymes (R*sa*I, *Hae*III, *Msp*I, *Hin*fI and *Alu*I) resulted in DNA fragment patterns that clearly demonstrate a match between adult and larval DNA, and thus these findings de facto confirm the larvae as instars of *T. mohelensis* (see Table 1). Reference slides of adults have been produced using Canada balsam after the specimens had been macerated in KOH, but larvae were embedded in Hoyer's mountant (see Ulitzka 2015). In the following, their features are designated according to Kucharczyk (2010). Microscopic examinations were carried out using

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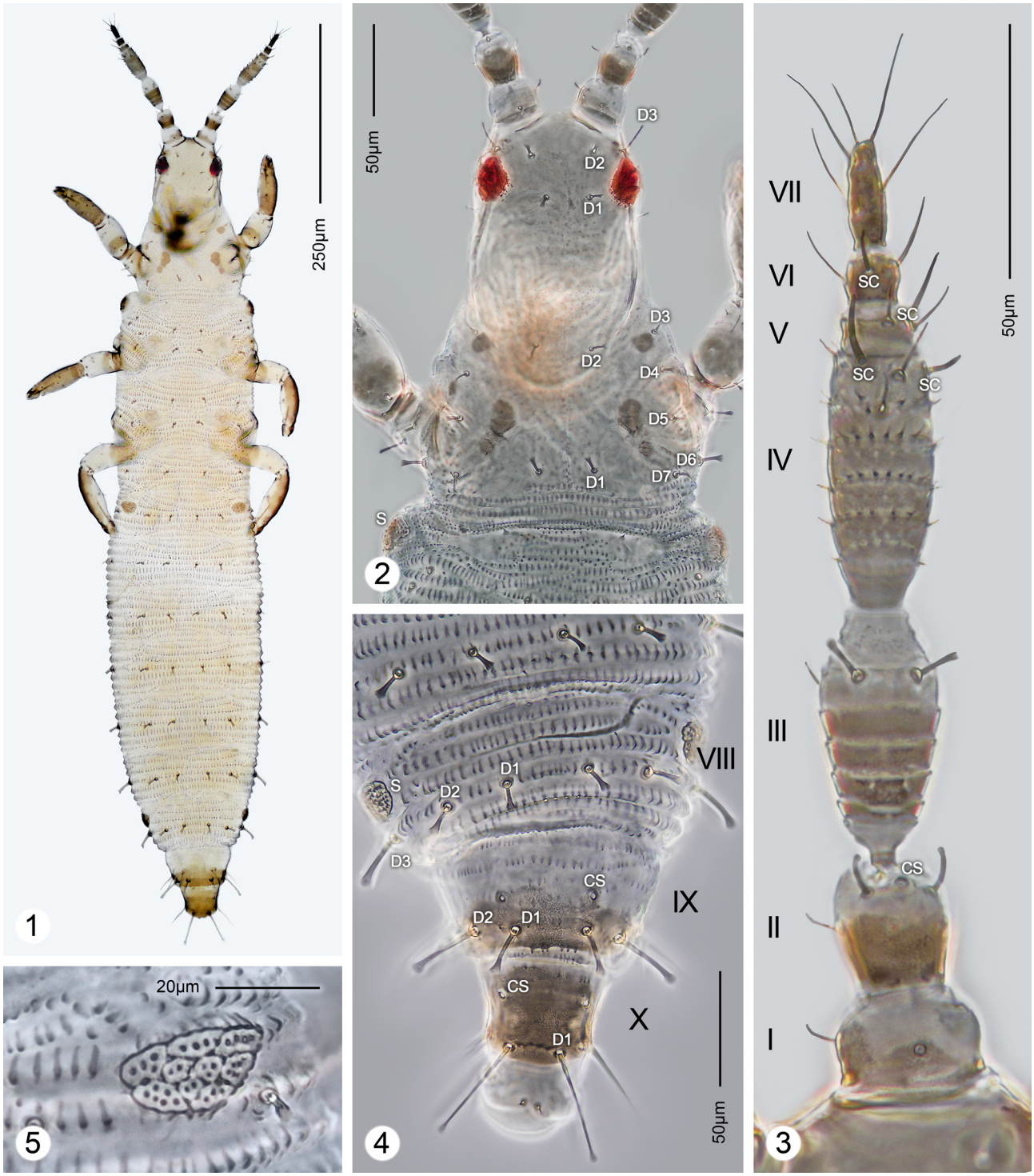
a Zeiss Stemi SV-11 Apo stereomicroscope and a Zeiss standard phase-contrast microscope. Photomicrography was performed with a digital camera attached to the microscopes (Canon EOS 70d). All images were produced in focus stacking technique with Helicon Focus software. Nik Sharpener Pro and Adobe Photoshop were used for final colour adjustment and sharpening.

T. mohelensis, female second instar larva (Figs 1–5). Body pale creamy yellow with well visible brown shadings on the legs as well as on the sclerotized plates of the pronotum and the distal parts of tergites IX and X (Fig.1); all plaques grey-brown as well as all setae and their points of insertion.



FIGURES 1–5. *Thermothrips mohelensis* Pelikán, second instar larva, female (D: dorsal setae, CS: campaniform sensillum, S: spiracle, SC: sense cone): **(1)** dorsal view (true colour); **(2)** head and pronotum (phase contrast); **(3)** right antenna (phase contrast); **(4)** abdominal tergites VIII–X (phase contrast); **(5)** right spiracle of abdominal tergite II (phase contrast).

Head (Fig. 2) 1.3 times as long as wide; dorsally with some scattered round plaques but without sclerotized plates except a slightly shaded area in front of stemmata; ventrally with brown shadings at tip of mouth cone, maxillary as well as labial palps completely grey-brown. Dorsal setae D1 attached at level of hind margin of stemmata and D2 at level of their front margin, D1 and D2 equal in length and shape, short with expanded tips; D3 attached just in front of stemmata, more delicate and much longer than D1 and D2, tips expanded; D4 minute. Stemmata with crimson pigmentation. Antennae (Figs 1, 3): segment I about twice as wide as long, slightly grey at base; II barrel-shaped, slightly longer than wide, brown in basal two-thirds, distally with 2 dorsal setae with expanded tips at a level slightly behind the campaniform sensillum; III about as long as I and II together, basally with a short pedicle, above pedicle annulated with 4 brown rings without microtrichia, distally with 2 dorsal setae with expanded tips; IV broadly attached to III, about as long as III, annulated with 6 rings, each distally with microtrichia, outer sense cone short, length about one third of inner; V shorter than width of distal rings at IV, outer sense cone slightly shorter than segment VII; VI about as long as wide with 1 outer sense cone; VII slender, about three times as long as wide; segments IV–VII completely grey-brown, VI and VII darkest.

Thorax: Pronotum (Fig. 2) smooth except peripheral parts, these covered with narrow plaques without microtrichia; with 5 pairs of small sclerotized brown plates (median plates forming a unit on each side); all pronotal setae short with expanded tips. Mesonotum and metanotum each with narrow plaques without microtrichia; without sclerotized areas on wing discs; with all setae short and expanded at tips; metathoracic spiracle shaded brown. Sterna sculptured with tiny plaques mainly arranged in rows but scattered in the middle of prosternum and mesosternum; sternal setae short and pointed, except metasternal V2, which are delicate and longer than width of femora. Legs: coxae and femora basally brown; tibiae brown on exterior margin; trochanters and tarsi completely brown.

Abdomen: Tergites II–VII with 7–8 rows of narrow plaques without microtrichia; setae short, about as long as the distance between the rows of plaques (D3 on caudal tergites slightly longer), expanded at tips. Tergite VIII (Fig. 4) with 5–6 rows of narrow plaques; setae D3 1.5 times as long as D2. Tergite IX (Fig. 4) with stronger sclerotization only in posterior part extending to level of campaniform sensilla; sculptured with 4–5 irregular rows of small plaques anterior to setae; posteromarginally with a row of minute irregular teeth and with a band of tiny plaques caudally to these teeth; distance of campaniform sensilla 1.4–1.6 times of distance between D1 setae. Tergite X (Fig. 4) completely sclerotized; sculptured at base with some irregular plaques in front of campaniform sensilla; setae D1 about as long as tergite X, expanded at tip (spatulate). Spiracles (Fig. 5) on II and VIII grey-brown; with 8–11 enlarged facets, each with up to 6 pores. Sternites with well developed sculpture similar to dorsal one but with plaques more rounded and much smaller; sternal setae V1 and V2 short with pointed tips and V3 slightly curved and blunt, except at sternite IX which has V1 short and pointed, V3 long and strong with a curved pointed tip and V2 lacking (distinctive feature of females, see Vierbergen *et al.* 2010), and sternite X which has long and pointed setae.

Measurements. Female MU-RU-01/11 in microns: Body length 1080. Head, length 132; width at base 98; setae D1 8, D2 7, D3 19, D4 5; distance between D1 24, D2 34. Pronotum, length 101; largest width 1560; setae D1 10, D2 10, D3 7, D4 7, D5 10, D6 14, D7 10; distance between D1 and D2 equal, each 31. Abdomen, length 653; largest width 226 (at segment IV). Abdominal tergite II, D1 7, D2 8, D3 10; spiracles, length 17, width 24. Tergite VIII, D1 12, D2 16, D3 24. Tergite IX, D1 24, D2 38; distance between D1 33; distance between campaniform sensilla 46; posteromarginal teeth 2–2,4. Tergite X, D1 48; distance between campaniform sensilla 36. Antennae, length 187; length (largest width) of segment I 19 (29), II 29 (24), III 48 (24), IV 48 (22), V 7 (14), VI 10 (10), VII 22 (7); sense cones, outer (inner) on segment IV 7 (19), V 19 (–), VI 14 (–).

***T. mohelensis, male* second instar larva.** Very similar to female but smaller, body length: 890. Abdominal sternite IX with setae V2 developed (distinctive feature of males, see Vierbergen *et al.* 2010).

Specimens studied. RUSSIA, East Siberia, Yakutsk, on a steppe slope in the botanical garden of the Institute for Biological Problems of the Cryolithozone, 8 females, 2 males with 1 male and 2 female second instar larvae, from *Galium verum*, 19.vi.2017 (TG Evdokarova leg.), in Ulitzka collection, MU-RU-01. 3 females, 2 males and 2 larvae (sex not identified) from the same location, used for ITS-RFLP analysis, 10.vi.2020 (TG Evdokarova leg.), from *Galium verum*.

Diagnosis. Even though *T. mohelensis* seems to be closely related to members of *Rubiothrips*, its larvae rather resemble to those of *Anaphothrips*-species. Abdominal tergite IX setae D1 are thickened and spine-like in *Rubiothrips*, whereas these setae are bristle-shaped in *Anaphothrips* (except *A. euphorbiae*) and *T. mohelensis* (Fig. 4). Furthermore, the structure of the spiracles, in having facets with pores, is nearly the same in larvae of *Thermothrips* and *Anaphothrips* species (with the exception of *A. euphorbiae*). Attempts at identifying second instars of *T. mohelensis* with keys provided by Priesner (1928) and Vierbergen *et. al.* (2010) result in *A. obscurus*. In fact, the larvae of both species are quite similar. However, they can be distinguished by the following character states:

- Head mediodorsally with a small rounded sclerotized plate in front of setae D2; dorsal setae on antennal segments II and III bluntly pointed; pronotum with 1 pair of small sclerotized plates just behind setae D3; abdominal tergite VIII with a regular row of posteromarginal minute teeth, X with setae D1 finely acute. Host plants: various grasses (Poacea) . . . . . *A. obscurus* (Müller)
- Head without a median sclerotization in front of setae D2 (Fig. 2); dorsal setae on antennal segment II and III expanded (Fig. 3); pronotum with 5 pairs of small sclerotized brown plates (Fig. 2); abdominal tergite VIII with an irregular row of posteromarginal minute teeth, X with setae D1 expanded at tip (spatulate) (Fig. 4). Host plants: *Galium verum* and *G. mollugo* (Rubiaceae) . . . . . *T. mohelensis* Pelikán

TABLE 1. Adult and larval DNA fragment patterns with various primer pairs and restriction enzymes.

	Adult 1	**Adult 2**	**Larva II**
Primer pair 28z/P1.			
PCR products (bp)	1628	1552	1546
Restriction enzyme		Fragments (bp)	
R*sa*I	281	345	356
*Hae*III	976	976	972
	230	253	242
*Msp*I	1350	1352	1339
Primer pair 18j/O1.			
PCR products (bp)	1904	1813	1775
Restriktion enzyme		Fragments (bp)	
R*sa*I		503	539
		459	488
		378	400
*Hae*III		389	397
M*sp*I	502	503	503
*Hin*fI	701	713	681
	632	648	619
Primer pair Cs249/Cs250.			
PCR products (bp)	1930	1892	1899
Restriction enzyme		Fragments (bp)	
R*sa*I	464	471	464
	441	444	437
	388	389	386
	363	364	358
*Hae*III	451	459	437
	281	289	276
	233	244	227
	182	189	177
	162	171	156
M*sp*I	447	462	439
	282	292	273
*Hin*fI	666	682	655
	574	584	563
	231	243	224
*Alu*I	1324	1341	1320
	576	580	569

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