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The Life Cycle of *Ctenothrips distinctus* (UZEL, 1895) (Insecta: Thysanoptera) and its Influence on the Host Plant *Convallaria majalis* L.

Halina KUCHARCZYK, Marek KUCHARCZYK and Dorota TCHÓRZEWSKA

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Original article

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In this paper, we describe the morphological characteristics of the preimaginal stages of *Ctenothrips distinctus*, as well as its life cycle in temperate climate conditions. We also revise the key characters of the second larval instar of *C. distinctus*, which were previously confused with those of *Taeniothrips picipes* (Zetterstedt, 1828). The morphological characteristics of the *C. distinctus* propupa and pupa represent their adaptations to moulting in the soil, not on the host plant. The study of the biology of *C. distinctus* is supplemented by an analysis of the impact of the foraging by this insect on its host plant *Convallaria majalis*. Based on the morphological, anatomical and histological analyses of Lily of the Valley leaves, we show that both adults and larval instars feed on the epidermal cells of both the upper and lower sides of the leaf blade in *C. majalis*. However, the assimilation parenchyma cells located immediately below the epidermis at the feeding site retain their shape.

Key words: thrips, pupae, larvae, Lily of the Valley, morphology, histology, feeding damage, biodiversity.

Halina KUCHARCZYK, Marek KUCHARCZYK, Department of Zoology and Nature Protection, Institute of Biological Sciences, Maria Curie-Skłodowska University. Akademicka 19, 20-033 Lublin, Poland.
E-mail: halina.kucharczyk@mail.umcs.pl
marek.kucharczyk@mail.umcs.pl
Dorota TCHÓRZEWSKA✉, Department of Cell Biology, Institute of Biological Sciences, Maria Curie-Skłodowska University. Akademicka 19, 20-033 Lublin, Poland.
E-mail: dorota.tchorzewska@mail.umcs.pl

Ctenothrips distinctus (Uzel, 1895) of the order Thysanoptera (Insecta) is one of the five species of the genus *Ctenothrips* in the family Thripidae. To date, this genus has only been recorded in the Northern Hemisphere (MOUND & HASTENPFLUG-VESMANIS 2021). *Ctenothrips* was described by FRANKLIN (1907), based on the species *C. bridwelli* collected in the Bellamy River swamps in New Hampshire (North-East USA). This genus was then revised, and the characteristics of *Ctenothrips* adults were described by WANG *et al.* (2020). The thirteen species described originally were reduced to five when they were synonymised, mainly with *C. distinctus* and *C. kwanzanensis* Takahashi, 1937 (CHEN 1979; FENG *et al.* 2003; HU & FENG 2011, 2013, 2014; XIE *et al.* 2011; XIE *et al.* 2013; TYAGI *et al.* 2014; CUI *et al.* 2018). *Ctenothrips* species have a Holarctic distribution: *C. bridwelli* is known to reside in the USA and Canada; *C. distinctus* resides in Europe and China; and three remaining species – *C. transeolineae* CHEN 1979, *C. parisae* WANG, LI, TONG & MOUND 2020 and *C. kwanzanensis* – reside in South-East Asia (Nepal, India, Korea, Japan, Taiwan and China) and Iran (MIRAB-BALOU 2021; THRIPS WIKI 2022). *Ctenothrips* species are herbivorous and they forage on the leaves of ferns and plants from different families, e.g. Asparagaceae, Melanthiaceae, Melastomataceae, Rubiaceae, Crassulaceae, Urticaceae and Lamiaceae, among others. They have also been collected from grasses (Poaceae) (XIE *et al.* 2013; WANG *et al.* 2020).

Ctenothrips distinctus was described in 1895 by UZEL as *Physopus distincti* from the Bohemian region of the Czech Republic. So far, this is the only member of the genus to reside in Europe (UZEL 1895;

VIERBERGEN 2021). As is the case with the majority of thrips species, the life cycle of *C. distinctus* and the morphology of its preimaginal stages are not yet known. The exception is the second instar larva, whose characteristics were presented in a key to the second instar larvae of the Thripidae of the Western Palearctic (VIERBERGEN *et al.* 2010). However, based on the features of the larva presented in this publication, we will suggest that the previous description was incorrect or related to a different species.

The host plant on which *C. distinctus* is found in Poland is *Convallaria majalis* L. (Lily of the Valley; Asparagaceae), which grows in pine and mixed forests, oak woods and in floristically poor forms of oak-hornbeam forests. The Lily of the Valley is not a rare or endangered species in most of its European range. However, *C. distinctus*, which feeds and breeds on this plant, is infrequently recorded in Poland. For this reason, it has been included on the red list of threatened thrips species in East-Central Poland in the category of LC (lower concern) (KUCHARCZYK & KUCHARCZYK 2008). *Convallaria majalis* is a valuable plant not only from the aspect of biodiversity, but also due to its medicinal properties: its above-ground parts contain numerous biologically active compounds, while the glycosides obtained from the herbs of this plant, e.g. convallatoxin and convallataxol, exhibit strong effects on cardiac activity and are used mainly in cases of heart failure (SIEBENHAAR 1956; STANSBURY *et al.* 2012). In addition, Lily of the Valley oil is used in aromatherapy and the cosmetics industry. All the parts of *C. majalis* contain about 40 types of glycosides, flavonoids and saponins. Cardiac glycosides, in particular, are often used by plants as a defence against herbivorous insects, but some species from several orders have become resistant, e.g. Danaidae, Arctiidae (Lepidoptera), Chrysomelidae, Cerambycidae (Coleoptera), Aphididae (Hemiptera), and Phyrgomorphidae (Orthoptera) (AGRAWAL *et al.* 2012), which indicates the high adaptation potential of these insects.

The aims of this research study were focused on two complementary aspects: 1) to analyse the life cycle of *C. distinctus* and the morphology of its preimaginal stages, in order to revise the description of the second instar larva given in the key created by VIERBERGEN *et al.* (2010); and 2) to carry out a comparative analysis of the morphological, anatomical and histological structures of Lily of the Valley leaves exposed to thrips, in order to characterise the specific changes in the structure of such leaves caused by the insect foraging. Therefore, a detailed knowledge of the biology of *C. distinctus* and an analysis of the effects of its foraging behaviour on *C. majalis* leaves constitute a holistic approach to this research topic. This is extremely important in light of the ongoing dynamic changes taking place in the environment, which are altering the distributions of both plant and animal species and are impoverishing biodiversity worldwide.

Materials and Methods

Plant and insect materials; rearing of thrips

The Convallaria majalis specimens were obtained from deciduous oak and oak-hornbeam forests in two locations in Poland: from the edge of the Kwiatówka Reserve (50°28′29″N, 20°09′25″E), Małopolska Upland (South Poland) in 2018; and from the vicinity of Końskowola Village (51°43′78″N, 22°03′01″E), Lublin Upland (South-East Poland) in 2019. At the beginning of May, leaves with *C. distinctus* feeding symptoms, and healthy leaves serving as a control material, were collected in the natural locations of this plant in the above-mentioned forests. Macroscopic images of the leaves with *C. distinctus* feeding symptoms were taken with a Nikon D300 camera equipped with a 60 mm AF MICRO NIKKOR lens.

In 2018, adult *C. distinctus* females and males were observed on the leaves collected in the forest. The insects were reared from 2 May to the end of June, when the eggs and first larval instars of the subsequent generation appeared. In 2019, the plants were collected on 11 May. No adults were found on the leaves, but there were traces of feeding, defecation and egg-laying. The insects were reared until 6 June, when the subsequent generation of adults appeared.

To breed the insects, leaves with signs of feeding were placed in plastic boxes (20 cm long x 10 cm wide x 7 cm high) and stored in the laboratory at 24°C for 16 h in light, and at 10°C for 8 h in the dark (LD 16:8), in phytotron chambers at the Department of Zoology and Nature Protection, Marie Curie-Skłodowska University, Lublin. Several layers of paper towels were placed on the bottom of each box. A wet towel kept the leaves fresh for a longer time and created good conditions for pupal development. Specimens of the different stages of development were successively selected and preserved in 70% ethanol. Before mounting, the specimens of the larvae and pupae were rinsed with distilled water and stored in lactic acid for 4-7days. Finally, after a brief rinse in glycerine, all the specimens of the preimaginal stages were mounted in Berlese fluid. The adults were mounted in Canada balsam, according to the procedure described by MOUND & KIBBY (1998).

Photographs of the life stages of *C. distinctus* were taken with the scanning microscope VEGA3 TESCAN (SEM HV: 30.00 kV) and with an Olympus BX 61. The measurements (in microns) were made and the characteristics were observed using differential interference contrast (DIC) and a computerised measuring system under an Olympus CellSens Dimension image analyser. The slide-mounted voucher specimens were deposited in the collection of the Department of Zoology and Nature Protection, Marie Curie-Skłodowska University, Lublin (Poland).

Plant analyses

Light Microscopy (LM)

For the anatomical analyses, *C. majalis* leaves with *C. distinctus* feeding symptoms were collected, and healthy leaves were used as the control material. The material was randomly sampled from 20 plants. The analyses were performed using fresh fragments of a manual cross-section and a fixed material. The leaf fragments were fixed in AA fixative (100% acetic acid, 96% ethanol at 1:3 v:v), then dehydrated in a series of ethanol concentrations of 70, 90, 96 and 100%, and were embedded in paraffin wax using the conventional methods (GERLACH 1972). 7-µm-thick sections were cut on the rotation microtome MICROM HM340. Next, the sections were stained with safranin, which stains lignified fragments red; and with light green, which stains the cytoplasm and cellulose cell walls green. Finally, the sections were examined under a Nikon Eclipse *N*i light microscope. The photographic documentation was prepared with a digital camera and the NIS-Elements BP software.

Scanning electron microscope (SEM)

Fragments of leaves with signs of feeding were collected for an analysis of their surfaces (from the top and bottom of the leaf lamina). They were fixed in a 5:5:90 (v:v:v) mixture of glacial acetic acid: formalin (40%): ethanol (70%) and were dehydrated in a graded acetone series (40%, 70%, 80% and 100%). Thereafter, the whole material was frozen in liquid nitrogen. The samples were analysed under a scanning electron microscope (LEO1430VP) with an accelerating potential of 15 kV.

Fluorescence microscope (FM)

To visualise the cuticle on the epidermis of the *C. majalis* leaves, hand-made cross-sections of healthy leaves were prepared. They were placed in a 0.02% auramine 0 solution in a TRIS buffer for several minutes (HESLOP-HARRISON & HESLOP-HARRISON 1980). After rinsing with distilled water, the slides were analysed under a fluorescence microscope Nikon Eclipse*N*i-u at an excitation wavelength of 330-380 nm and an emission wavelength of over 480nm (UV). The photographic documentation was prepared with a digital camera and the NIS-Elements BP software.

Results

Life cycle of *C. distinctus*

In nature, the adult insects leave the soil and start to feed on leaf blades during the development of *C. majalis* leaves in the spring (April-May). After a few days, the females lay eggs between the epidermal cells on the lower surface of the leaves. The hatching first instar larvae and second instars feed on the leaves by sucking out the contents of the epidermal cells. In the experimental conditions of the laboratory environment, we observed the second instar larvae migrating between the layers of the damp towels, where they continued their development into the propupal and pupal stages. Compared with the mobile feeding larvae, the experimental pupae represented mostly quiescent non-feeding stages, which in natural conditions probably develop in the soil. In the experimental conditions, the whole life cycle (from the egg to the newly-emerged generation of adults) took about four weeks to complete. The first larval instar was the shortest stage lasting only 1-3 days; the second larval instar fed for 10-14 days; the propupae developed for 2-4 days; and finally, the pupal stage lasted 7-10 days. Metamorphosis took place during the latter two stages, after which the pupae finally matured into the adult form (Fig. 1).

Morphology of the adults of *C. distinctus*

The macropterous adults have a reticulated sculpture on the thorax (except the pronotal median part), on the dorsal and ventral sides of the abdomen, and on the lateral sides of the head. The head is constricted behind the eyes (Figs 2A, B, C, D, F; 3A, B, C, D). The legs are bicoloured with dark brown coxae and femurs, and with yellow tibiae and tarsi; the antennal segments I, II and VI-VIII are darker than III-V, which are yellow. Both females and males lack the meso- and metasternal spinula (Fig. 2E), and the hind margin of abdominal segment VIII bears a regular comb (Fig. 3A). The males possess biscuit-formed (narrower in the middle part) areae porosae on sternites III-VII (Fig. 3C). All setae, on both the dorsal and ventral sides of the body, are acute (Figs 2C, D; 3A, B, C, D).

Morphology of the preimaginal stages of *C. distinctus*

First larval instar (Fig. 4)

Body is brilliant white, not sclerotised. Cuticle is smooth with short microtrichia situated irregularly on the dorsal side of the meso-, metanotum and abdomen. Antennae: segments I-IV are with the external longest setae knobbed at the apex; IV-VII are jointed, tapering towards the apex (Figs 4A, B). Abdomen: segment VIII is without a dorsal comb; most dorsal setae are knobbed except the longest pair on segment X, which is acute (Figs 4C, D, E).

Measurements (in microns): body length – 1250, antennal length – 225. Dorsal setae on the head: D1 25, D2 45, D3 22.5, D4 50; distance D1-D1 37,5, D2-D2 52. Antennal segments: I 25, II 30, III 45, IV

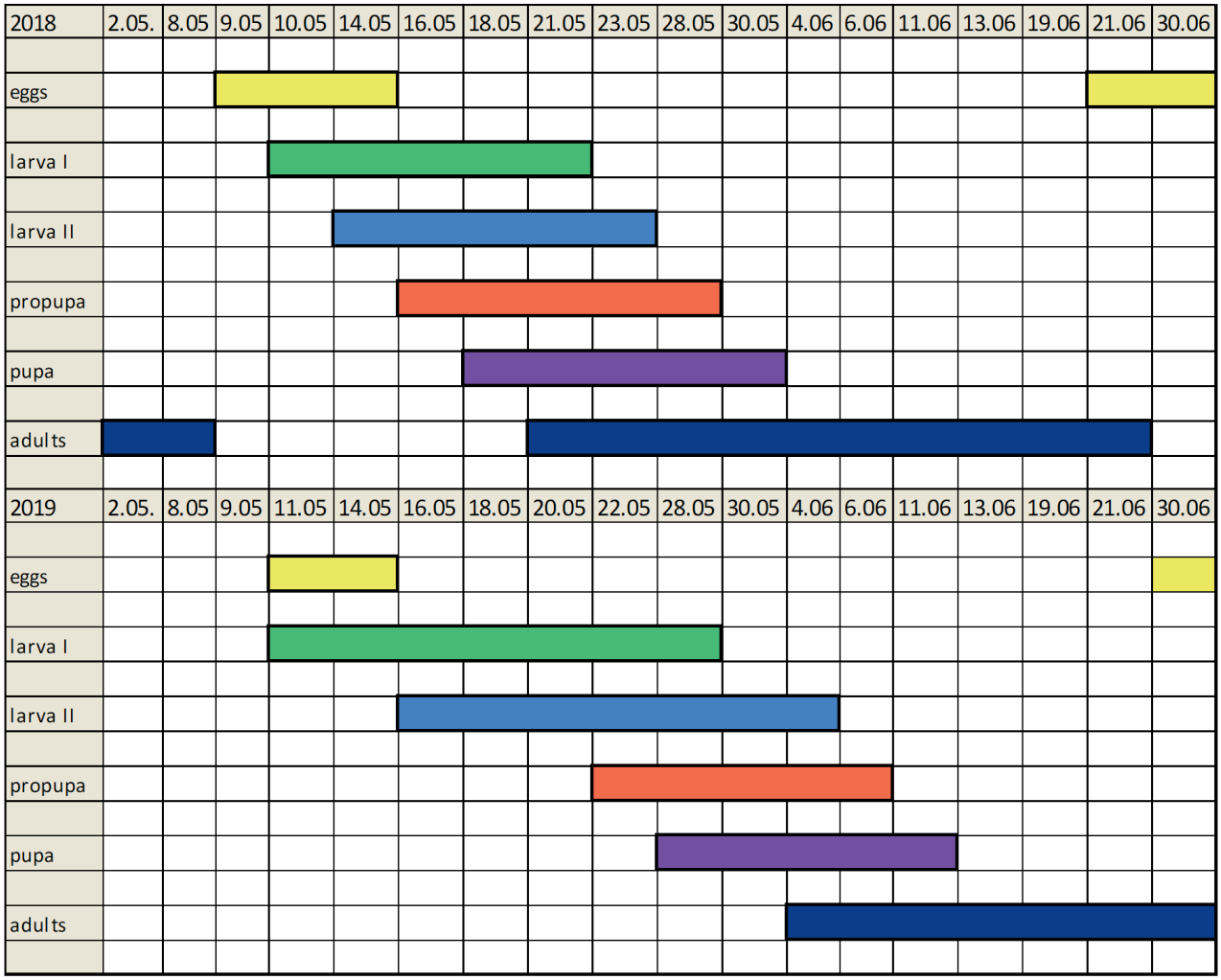

Fig. 1. Life cycles of *C. distinctus* in 2018 and 2019.

55, V 10, VI 15, VII 40. Dorsal pronotal setae: D1 25, D2 30, D3 20, D4 17.5, D5 40, D6 45; distance D1-D1 62.5, D2-D2 67. Length of abdominal setae on the tergites: IV - D1 20, D2 30, D3 35; distance D1-D1 87.5, D1-D2 50; V - D1 20, D2 30, D3 35; distance D1-D 95, D1-D2 57.5; VIII - D1 25, D2 32.5, D3 35; distance D1-D1 72.5, D1-D2 42.5, and IX D1 45, D2 60; distance D1-D1 40; distance between campaniform sensillae on tergite IX 40. Length of abdominal seta on tergite X D1 65. Spiracle on abdominal segment II: length/width 7.5/17.5; number of facets 8-9.

Second larval instar (Fig. 5)

Body is pale yellow or creamy, head with two round sclerotised plates near the eyes, smooth. Antenna: segments III-VII darker than I and II (Figs 5A, B). Abdominal segments IX and X darker in 2/3 of the distal part (Fig. 5E). Pterothorax and abdominal tergites (without segments IX and X) are with 8-9 transverse rows of plates lacking microtrichia (Figs 5A, C, D, F). Dorsal site of segment IX is with three rows of short microtrichia forward of the D1 setae (Fig. 5G). Abdominal sternites are without plates in the middle part – between the V1 setae (Fig. 5F). Abdominal segment VIII is surrounded by short teeth (Figs 5E, G). Dorsal setae on the head, thorax and abdomen are knobbed, with the ventral ones acute.

Measurements (in microns, min-max of 10 measured specimens): body length 1700-1900; head length with the mouth cone 265; antennae length 290-312. Head dorsal setae: D1 40-44, D2 45-52.5, D3 32.5-37, D4 57.5-62; distance D1-D1 42-45, D2-D2 50-55. Antennal segments: I 30-37, II 40-47, III 65-72, IV 75, V 12.5-13, VI 20-23.5, VII 42.5-47.5. Pronotal setae: D1 37.5-45, D2 52.5-60, D3 40-47.5, D4 52.5-57, D5 55-60, D6 62.5-70, D7 47.5-57; distance D1-D1 68-70, D2-D2 72-80. Length of abdominal setae on the tergites: IV - D1 40-42.5, D2 47.5-57, D3 54-55;

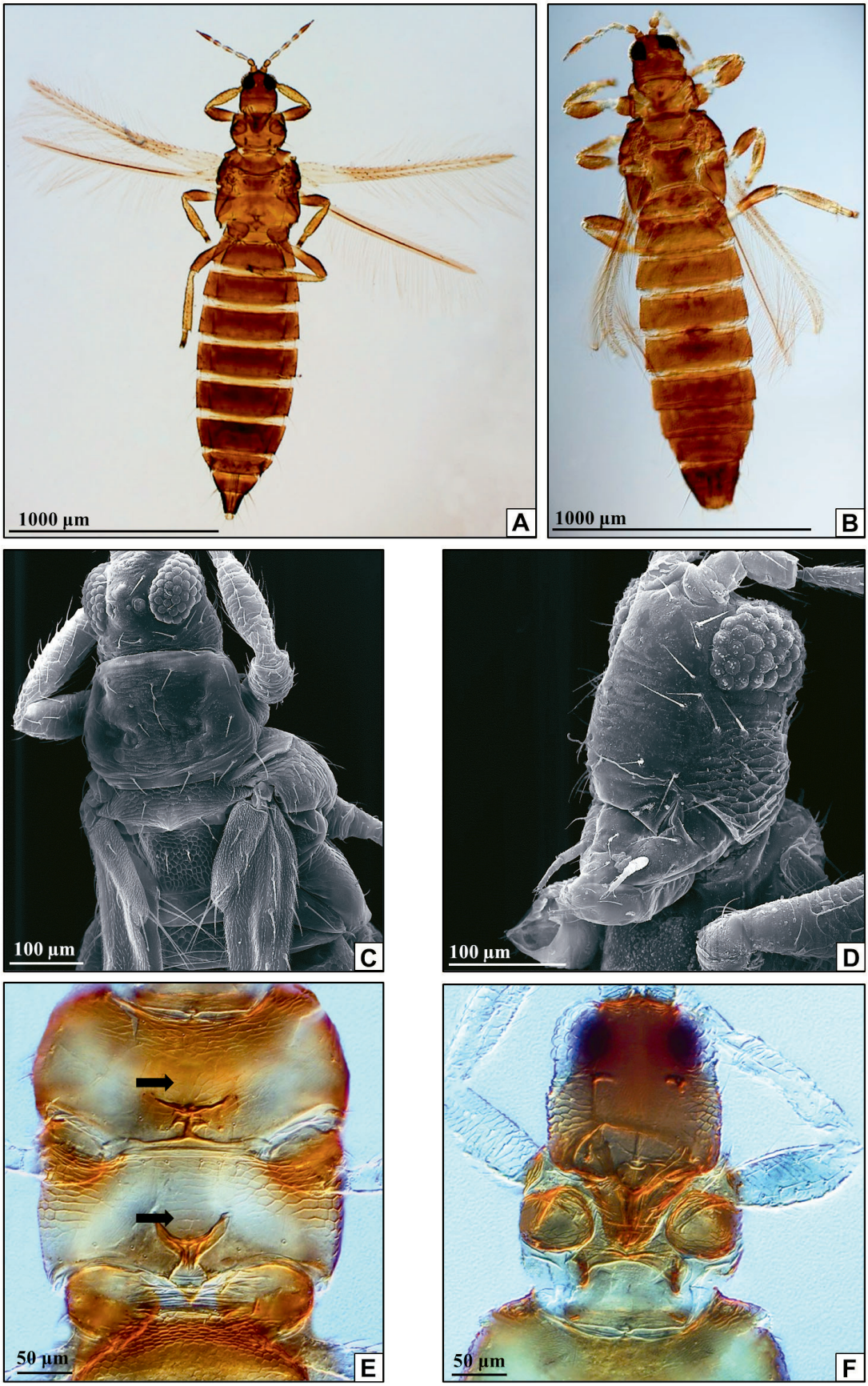

Fig. 2. Morphology of *C. distinctus* adults. A – female; B – male; C – head and pronotum; D – mouth cone; E – meso- and metasternum without spinulae (arrows); F – ventral side of the head and prothorax.

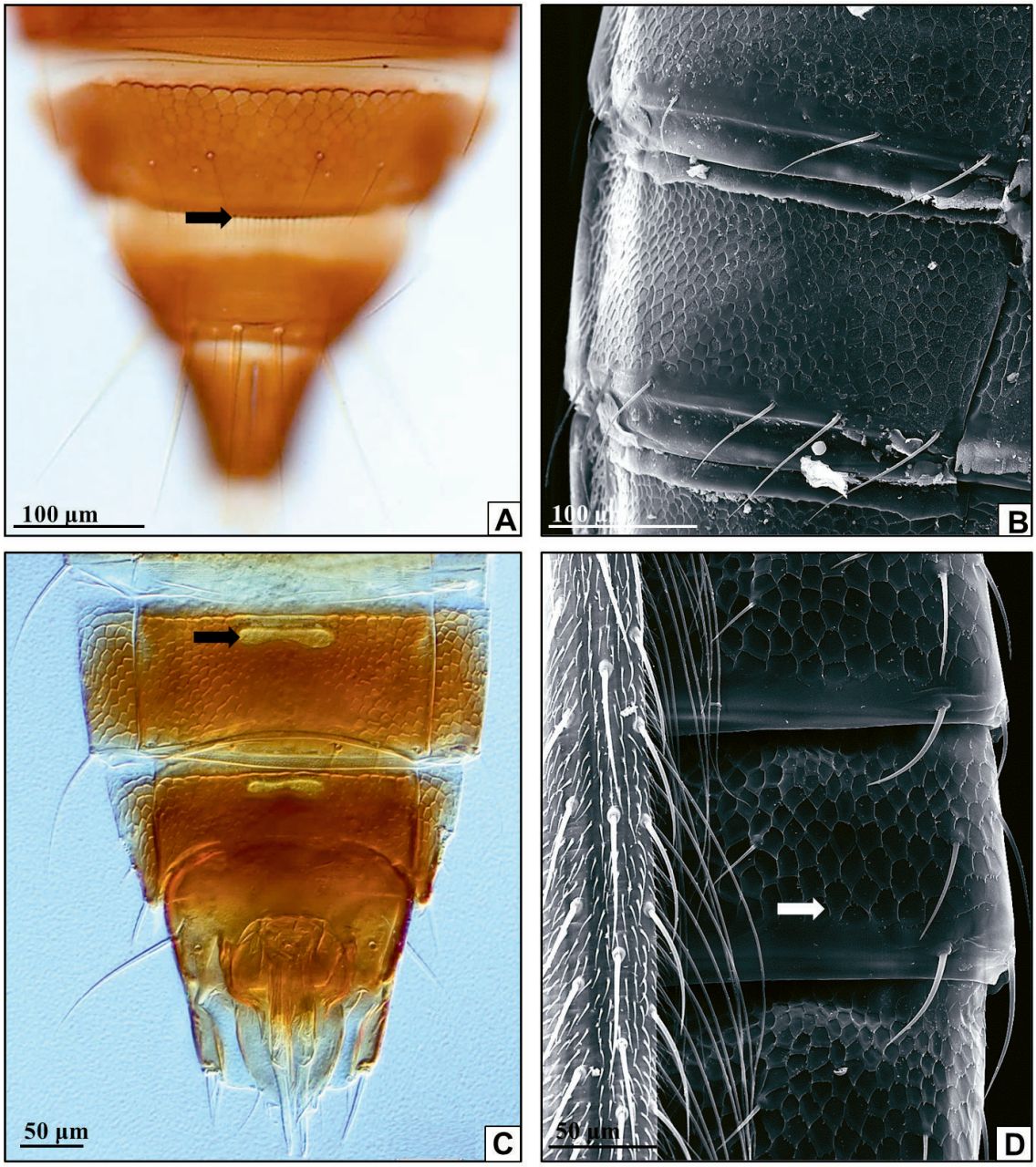

Fig. 3. Morphology of *C. distinctus* adults. A – abdominal tergites VIII-X, marginal comb of tergite VIII (arrow); B – sculpture of the abdominal sternites; C – male – abdominal sternites VII-X with areae porosae (arrow); D – sculpture of the abdominal tergites (arrow).

distance D1-D1 100-116, D1-D2 58-69; V - D1 45-46, D2 50-54, D3 55-60; distance D1-D1 100-114, D1-D2 50-70, VIII - D1 60-70, D2 60-62, D3 64-67.5, distance D1-D1 70-80, D1-D2 51.5-60; IX - D1 60-67.5, D2 70-75; distance D1-D1 57.5-60; distance between campaniform sensillae on tergite IX 60-62; length of the comb teeth 5. Spiracle on abdominal segment II length/width 14-16/ 27.5-30; number of facets 10-13.

Revision of the second larval instar characteristics

We realised that the larva described and photographed under the name of *C. distinctus* by VIERBERGEN *et al.* (2010) was in fact *T. picipes*. In the publication by VIERBERGEN *et al.* (2010), Fig. 247 shows a row of 7-9 teeth between the setae D1 on the edge of segment IX, and Fig. 248 shows a more fully chitinised oval area between setae V1 on segment X.

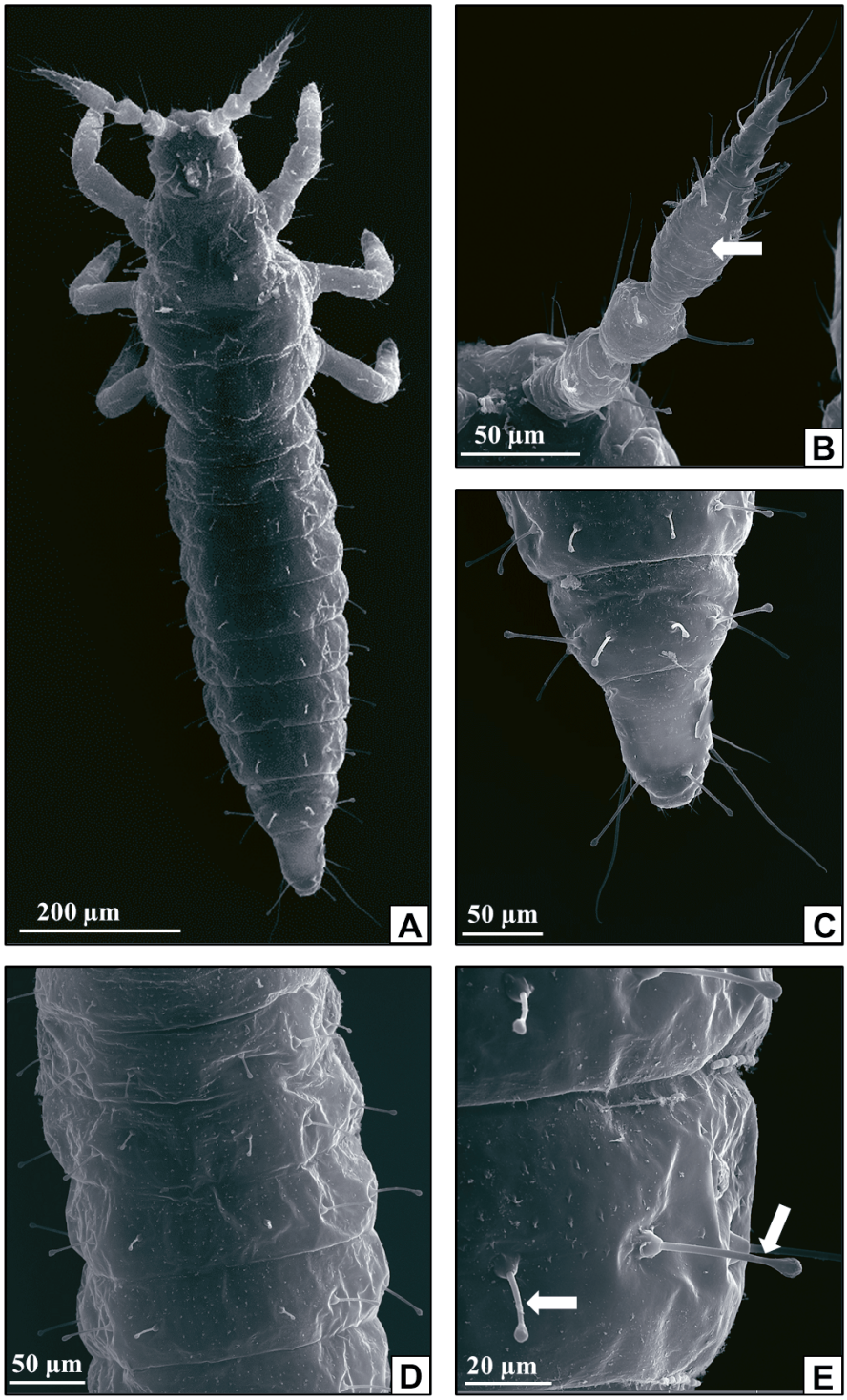

Fig 4. Morphology of the first larval instar. A – dorsal side of body; B – antenna: antennomeres 4-6 closely joined (arrow); C – abdominal tergites VIII-X; D and E – knobbed setae on the abdominal tergites (arrows).

These characteristics were different in the larva described in the present research. There was no plate on sternite X; in addition, the number of teeth between the D1 setae was 12-14, and their length was 5 µm. Previously, it was also found that living larvae of *T. picipes* were rose-red, whereas the *C. distinctus* larvae analysed in the present study were creamy in colour.

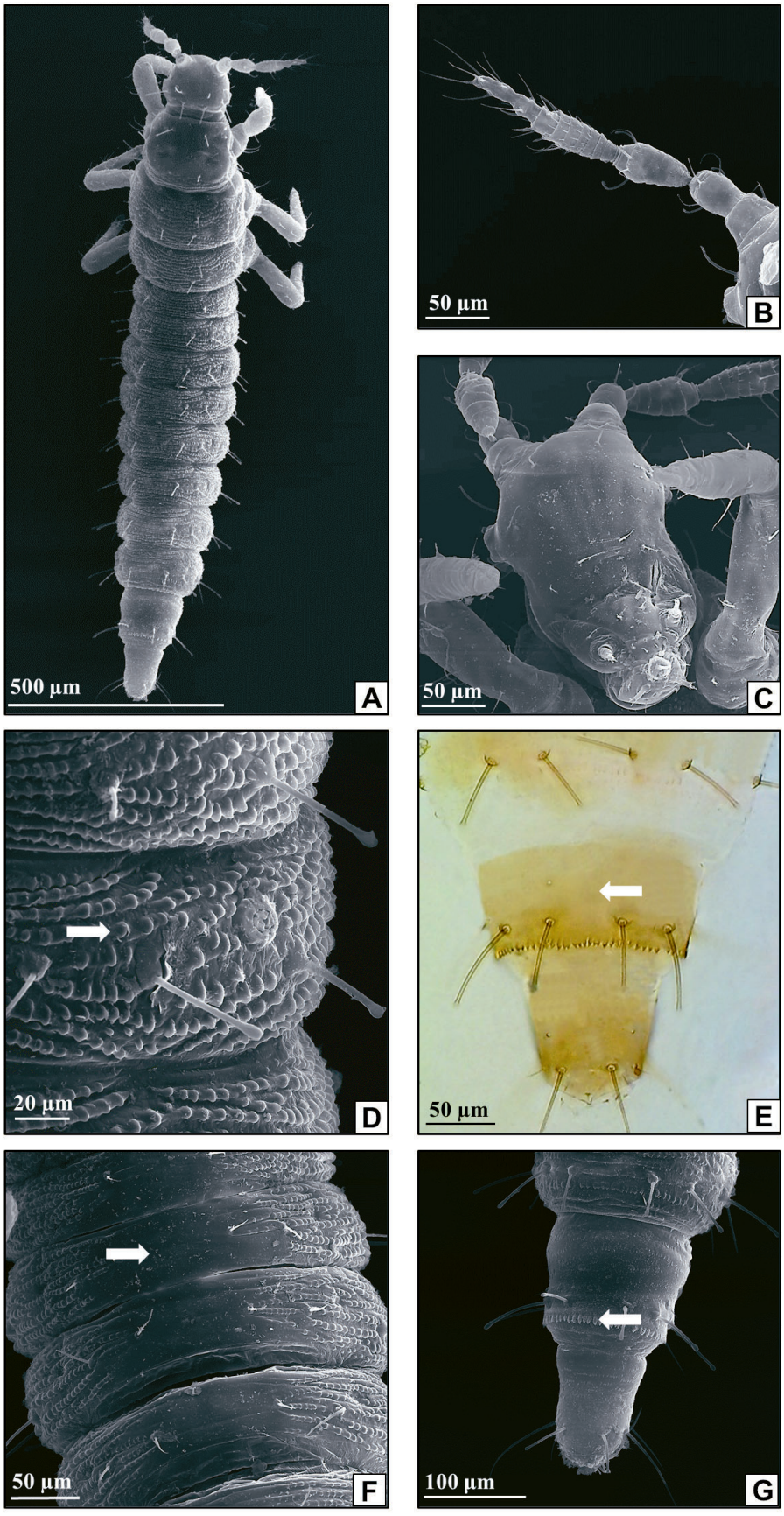

Fig. 5. Morphology of the second larval instar. A – dorsal side of body; B – antenna; C – ventral side of head with mouth cone; D – sculpture of the abdominal tergites (arrow); E – abdominal tergites VIII-X, chitinisation (arrow); F – ventral side of the abdomen, without sculpture in the middle part of the sternites (arrow); G – comb surrounding abdominal segment IX (arrow).

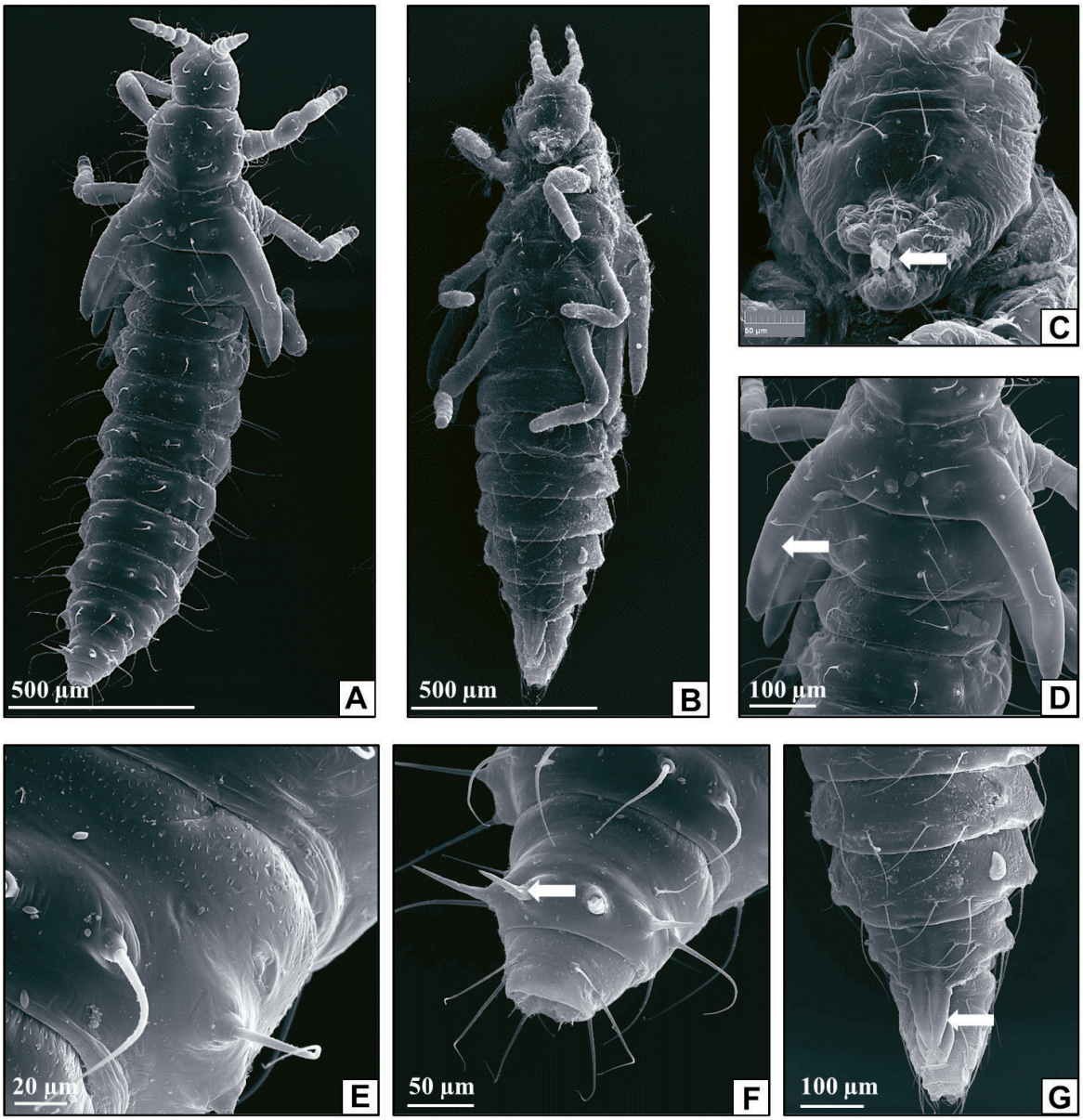

Fig. 6. Morphology of the propupa. A – dorsal side of body; B – ventral side of body; C – reduced mouth cone (arrow); D – wing pads, fore one (arrow); E – sculpture and setae of abdominal tergites VIII-IX (lateral part); F – dorsal side of abdominal tergites VII–X with thorns (arrow); G – female abdominal sternites with the ovipositor pads (arrow).

Propupa (Fig. 6)

Body is creamy and transparent. Head is shorter and wider than in the second larval instar, mouthparts are reduced, ocelli are absent (Figs 6A, B, C). Antennae: indistinctly segmented and shorter, pointing forwards. Pterothorax: wing pads (absent in the larvae) extended back to the second abdominal segment (Figs 6A, B, D). Abdominal epidermis are smooth except for abdominal segments VIII and IX, which have short microtrichia (Fig. 6E). Number and position of body setae is the same as in the second larval instar, but much longer and acute or filiform at the apex. Dorsal setae D1 and D2 on abdominal tergite IX are in the form of thorns (Fig. 6F). In females, there are two elongated genital lobes on the ventral site (Fig. 6G).

Measurements (in microns): body length – 1075, antennal length – 235. Head dorsal setae: D1 65, D2 50, D3 25, D4 100; distance D1-D1 30, D2-D2 35. Antennal segments: I 45, II 40, III 30, IV 35, V 85. Dorsal pronotal setae: D1 110, D2 150, D3 75, D4 85,

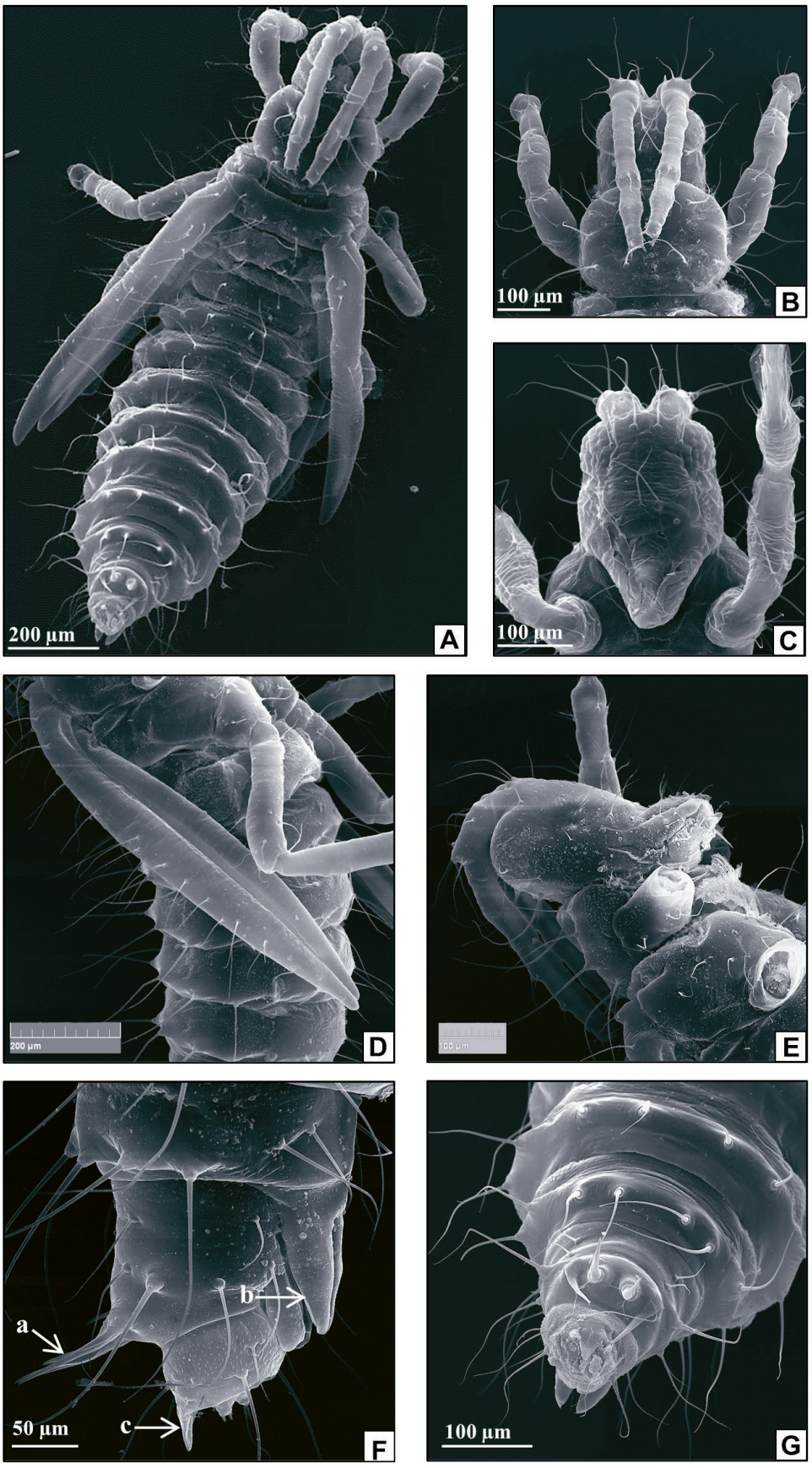

Fig. 7. Morphology of the pupa. A – dorsal side of body; B – head with the antennae and pronotum; C – ventral side of the head with reduced mouthparts; D – wing pads; E – lateral side of the head with mouth cone and antennae; F – female - lateral side of abdominal segments VII-X, a – thorns, b – ovipositor pads, c – apical projection; G – male - distal part of the abdomen.

D5 130, D6 105, D7 110; distance D1-D1 75, D2-D2 90. Length of abdominal setae on the tergites: IV - D1 85, D2 115, D3 145; distance D1-D1 87.5, D1-D2 50, V- D1 115, D2 135, D3 35; distance D1-D1 95, D1-D2 57.5; VIII - D1 110, D2 135-150, D3 160. Length/width of the abdominal thorns on tergite IX: D1 50/10, D2 60/10, thorns stout, distance D1-D1 45; distance between the campaniform sensillae on tergite IX 50. Length of abdominal setae on tergite X D1 65. Spiracle on abdominal segment II – length/width 7.5/17.5; number of facets 8-9. Length of the wing pads: I pair 285-375, II pair 220-265.

Pupa (Fig. 7)

Body is creamy, not sclerotised, epidermis smooth, except segment X, which is covered with short microtrichia. Head is longer than wide, mouthparts nonfunctional; mandible, maxillae and palps develop at the end of metamorphosis (Figs 7C, E). Antennae: segmented and pointing backwards as far as the fore or near the hind margin of the prothorax. Pterothorax: elongated wing pads extended backwards to the fourth or fifth abdominal segment (Figs 7A, B, D, E). Number and position of setae on the body are the same as in the second larval instar and propupa, but much longer, with acute or filiform apices (Figs 7A, B, C, D, E, F, G). Setae D1 and D2 on abdominal tergite IX are thorn-like (Figs 7F, G). In females, two elongate genital lobes present on the ventral side (Fig. 7F), longer than in the propupae, segment X with a blunt projection at the apex (Fig. 7F).

Measurements (in microns, female): body length – 2175, antennae length – 370. Head dorsal setae: D1 15, D2 125, D3 55, D4 125; distance D1-D1 50, D2-D2 25. Antennal segments: I 45, II 40, III 30, IV 35, V 85. Dorsal pronotal setae: D1 165, D2 165, D3 100, D4 125, D5 165, D6 155, D7 160; distance D1-D1 105, D2-D2 130. Length of abdominal setae on the tergites: IV - D1 170, D2 200, D3 215; distance D1-D1 140, D1-D2 85, V - D1 160, D2 175, D3 200; distance D1-D1 145, D1-D2 75, VIII - D1 165, D2 200, D3 200; distance D1-D1 90, D1-D2 40. Length/width of the abdominal thorns on tergite IX: D1 65-75/15-25, D2 80-90/15-20; thorns stout, distance D1-D1 10-15, D1-D2 20. Distance between campaniform sensillae on tergite IX 50. Apical projection on segment X: length/width 25-35/ 20-25. Spiracle on the abdominal segment II – length/width 7.5/17.5; number of facets 8-9. Length of the wings pads: I pair 560-600, II pair 480-500.

Morphology and anatomy of the *Convallaria majalis* leaves (Fig. 8)

The analysed *C. majalis* leaves had a lanceolate to an elliptical shape with the entire margins and arcuate venation converging at the apex (Fig. 8A). The epidermis is visible in the cross-section of a healthy leaf blade (control leaf, without foraging symptoms) consisted of thin-walled rectangular cells. The epidermis on both sides of the leaf blade had stomata with small air chambers underneath. The undifferentiated assimilation parenchyma, which was composed of oval cells relatively closely adjacent to each other, was visible beneath the single-layered epidermis (Fig. 8B). The vascular bundles in the *C. majalis* leaf were surrounded by relatively large parenchyma cells, and the xylem and phloem were situated in the centre. The vascular bundles on both sides were reinforced with a layer of sclerenchyma fibres with varying degrees of lignification of the cell wall, and this supporting tissue formed strands extending to the upper and lower epidermis (Fig. 8C). The analysis of the peeled epidermis of the *C. majalis* leaves showed the same structure of the cuticle on both leaf blade surfaces (Figs 8D, E).The cuticle had the same thickness on both the upper (Fig. 8F) and lower epidermis (Fig. 8G), and on the leaf veins (Fig. 8H). All the vascular bundles in the leaves exhibited layers of supporting tissue reaching the upper and lower epidermis (Figs 8H-D).

Morphology and anatomy of the *C. majalis* leaves with foraging symptoms (Fig. 9)

The plant was cultivated in the presence of insects and the traces of foraging, i.e. leaf blade necrosis and *C. distinctus* faeces, were analysed; importantly, the traces of foraging were visible on the upper (Figs 9A, B) and lower (Figs 9C, D) surfaces of the leaf blades. In both cases, the leaf veins were intact (Figs 9B, D). The SEM observations of the leaf surface at the foraging site showed that the epidermis layer was destroyed and the foraging area was delimited by the leaf veins (Figs 9E, F). The cross-sections of the *C. majalis* leaves at the foraging sites showed damage only to the epidermis layer on both the upper (Fig. 9G – marked with parenthesis) and lower (Fig. 9H – marked with parenthesis) surfaces of the leaf blade. The parenchyma cells located immediately below the epidermis at the foraging site retained their shape (Figs 9I, J). In addition, there was no damage to the assimilation parenchyma (mesophyll).

Discussion

Ctenothrips distinctus belongs to the family Thripidae of the order Thysanoptera. Like most species of this family, it feeds on plant cells by ingesting their contents. The adults and both larval instars feed on the same host plant, on which the eggs are laid. In Poland, *C. distinctus feeds* on *C. majalis*, which is currently the only plant host of this species known in the country. Moreover, this thrips has been reported in only a few localities in Poland; thus, it can be regarded as a rare species (KUCHARCZYK & KUCHARCZYK 2008). In Europe, *C. distinctus* has been recorded in Norway, Sweden, Germany, Hungary and Romania. *C. majalis* is usually the host plant of the insect species (zur

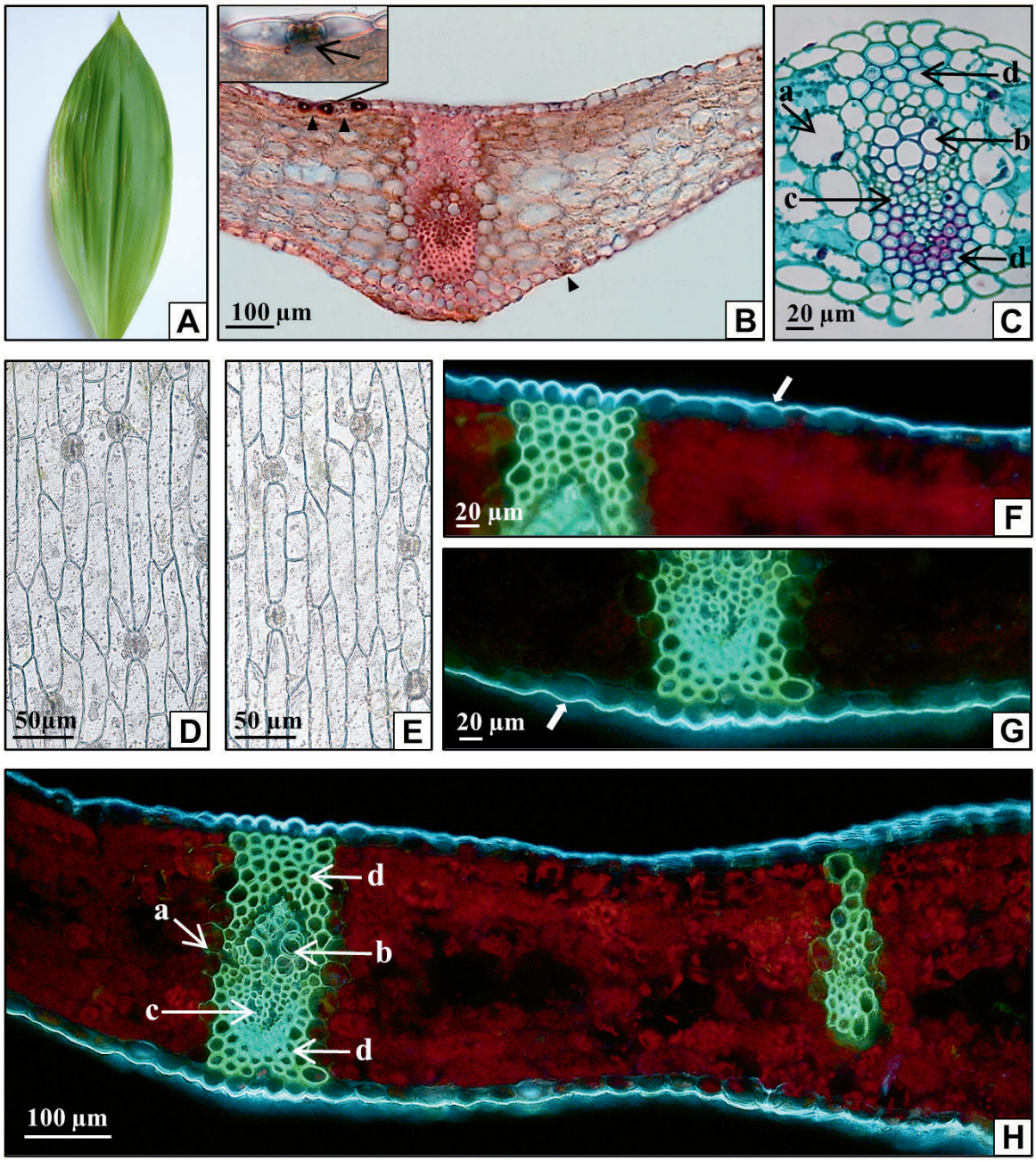

Fig. 8 *C. majalis* leaf (control). A – single leaf (macroscopic image); B – cross-section of the leaf blade and the midrib; stomata on the upper and lower sides of the leaf blade (arrowheads); small picture – cross-section of a stoma with a visible air chamber (arrow); C – cross-section of the vascular bundle: a – parenchyma around the vascular bundle, b – xylem, c – phloem, d – sclerenchymatous fibres; D – epidermis from the upper side of the leaf blade; E – epidermis from the lower side of the leaf blade; F – cuticle (arrow) on the epidermis of the upper side of the leaf blade; G – cuticle (arrow) on the epidermis of the lower side of the leaf blade; H – fragment of the leaf with vascular bundles: a – parenchyma around the vascular bundle, b – xylem, c – phloem, d – sclerenchymatous fibres (B-E: LM; F-H cross-section; auramine staining; fluorescence microscope).

STRASSEN 2003); additionally, this thrips has been frequently found on the leaves of *Polygonatum verticillatum* (Asparagaceae) (VIERBERGEN 2022; VIERBERGEN *et al.* 2010).

To date, there have been no detailed references to the *C. distinctus* life cycle and the morphology of its preimaginal stages. Additionally, no effects of its feeding on the host plant have been investigated, leaving the issue of the host-pathogen interaction unexplored. Therefore, the observations of all the developmental stages of *C. distinctus* and the impact of this insect on the host plant represent the first com-

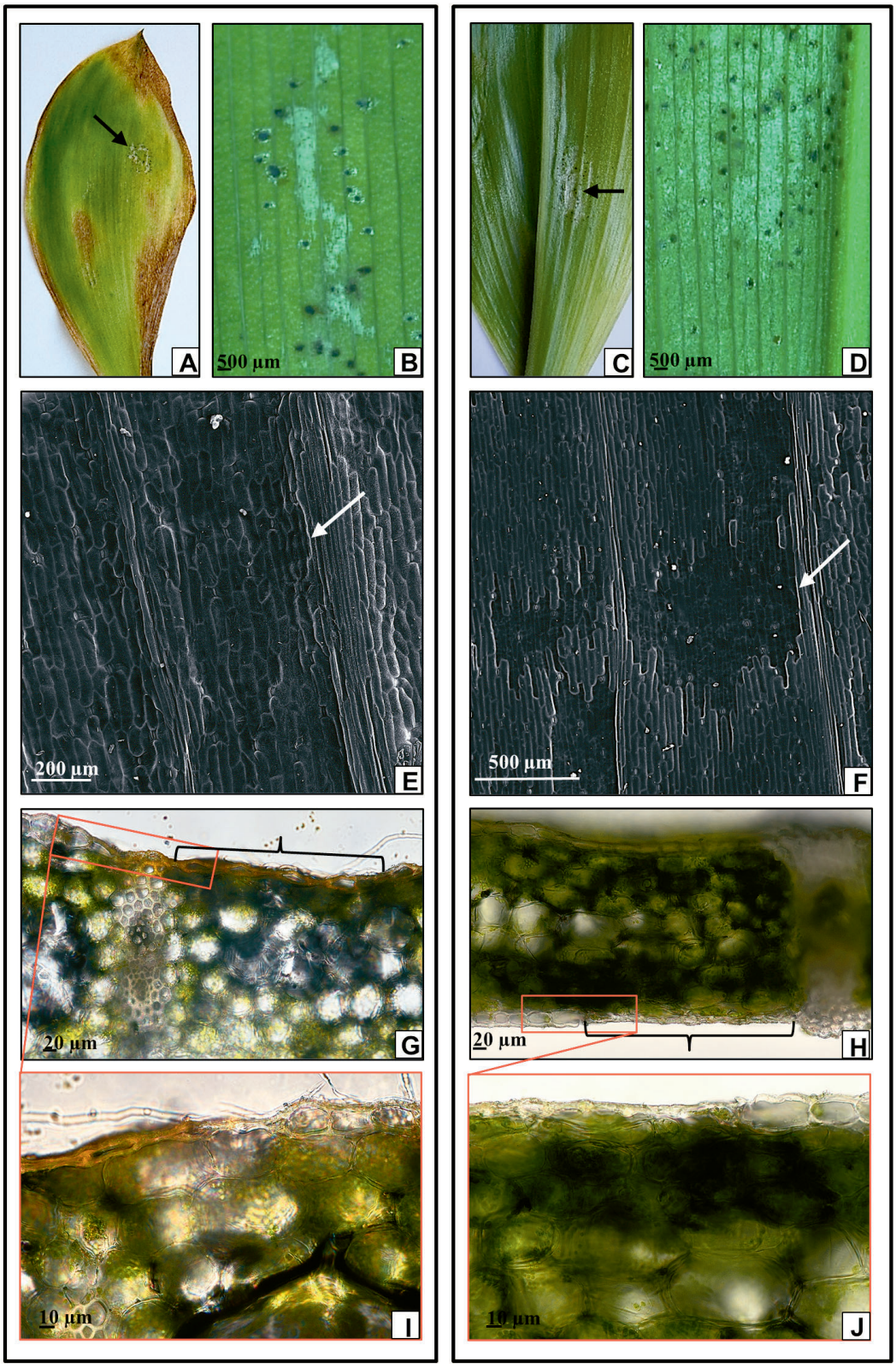

Fig. 9 *C. majalis* leaf with *C. distinctus* foraging symptoms: A – upper side of the leaf blade with the foraging area (arrow); B – fragment of the leaf with visible necrosis and insect faeces; C – lower side of the leaf blade with the foraging area (arrow); D – fragment of the leaf with visible necrosis and insect faeces; E – upper side of the leaf blade with the foraging site; F – lower side of the leaf blade with the foraging site; G – fragment of the epidermis on the upper leaf blade damaged by foraging (marked with parentheses); H – fragment of the epidermis on the lower leaf blade damaged by foraging (marked with parentheses); I – magnification of the fragment of the adjacent *C. distinctus*-damaged and non-damaged epidermis areas shown in photo G; J – magnification of the fragment of the adjacent *C. distinctus*-damaged and non-damaged epidermis areas shown in photo H (E-F: SEM; G-J – cross-section of the leaf; hand-made preparation; light microscope).

plex approach in investigations of the life cycle of *C. distinctus*.

It was found that the life cycle of this species takes about four weeks to complete, and is similar to that observed for *Thrips nigropilosus* UZEL 1895 investigated on *Mentha x piperita* in comparable experimental conditions (KUCHARCZYK *et al.* 2019). It should be underlined that little is known about the morphology of the preimaginal stages of most terebrantian species (MIYAZAKI & KUDO 1986; NAKAHARA & VIERBERGEN 1998; KOBRO 2002, 2011; KUCHARCZYK 2003, 2010; VIERBERGEN *et al.* 2010; KUCHARCZYK & KUCHARCZYK 2013a, b; XIE *et al.* 2013). So far, the characteristics of the larvae and pupae have been described only in *Ctenotrips yangi (*XIE *et al.* 2013). This species that was recorded for the first time from China was later synonymised with *C. kwanzanensis* by WANG *et al.* (2020). The authors described the main characteristics of the first larval instar: body length and colour and the antennal length. The described body size values were larger than those in the *C. distinctus* specimens investigated in our report (body length of 1250 versus 806 µm), but the latter value was somewhat similar (antennal length of 225 versus 212 µm). The morphological characteristics of the second instar larvae of both species were similar: the sculpturing of the meso- and metanotum and abdominal tergites I-VIII was clearly visible, and consisted of rows of plates devoid of microtrichia. The middle parts of the pronotum, sternites and abdominal segments IX and X were smooth. All the dorsal and lateral setae were knobbed, in contrast to the ventral setae, which were acute at the apex. The chitinisation of segments IX and X in *C. distinctus* extended to two-thirds of their lengths, but in the species described by XIE *et al.* (2013) it ended at the bases of the distal setae. Besides this data, the morphological characteristics of the pupal stages in most thrips species have not yet been investigated. So far, only KUCHARCZYK & KUCHARCZYK (2013b) have described the eco-morphological adaptation of the pupal stages of some species found in Poland. It has been reported that these stages of the species developing in grass inflorescences, or in the narrow crevices of leaf sheaths, have shorter setae and smaller abdominal thorns than the species developing on leaf blades or in flowers. Both the propupa and pupa of *C. distinctus* were found to have rather long, robust thorns on the abdominal segment IX. These structures may act as a defence against predators living in the soil, which is where this stage develops into the adult form. Furthermore, in laboratory conditions, most of the pupal specimens were collected from the bottom of the breeding boxes (covered by paper towels), which may suggest that in nature these stages develop in the soil near their host plant. KUCHARCZYK & KUCHARCZYK (2013b) also noticed differences between the sex forms in the pupal stage: the females were longer and had an abdominal apical projection, which was either blunt or acute at the apex, whereas the males had no such structure. The tip of their abdomen was rounded and had shorter bristles KUCHARCZYK & KUCHARCZYK (2013b). The descriptions of the *C. distinctus* pupal stages provided in the present study are the first such detailed reports. It should be pointed out that in the earlier study conducted by XIE *et al.* (2013), a general description was given of the propupa and pupa of *Ctenothrips yangi (*synonymised with *C. kwanzanensis*) that is found at high elevations in the Yunnan Mountains in China. The authors regarded this species as an alpine taxon. The comparison of the main morphological features (lengths of body, antennae and fore wing) of the Chinese and Polish specimens of propupae and pupae showed differences between both stages. Compared with the Polish specimens, the Chinese propupa had a longer body and fore wings and shorter antennae, whereas the pupa had a shorter body and fore wings. The antennal length was similar in the pupae from both regions.

In addition to a detailed description of the *C. distinctus* life cycle, the impact of this insect on the host plant *C. majalis* was investigated. This small perennial Asparagaceae plant grows up to 25 cm high, and its assimilation leaves have a relatively large surface. All parts of *C. majalis* are poisonous, and research on this herb has shown that toxic cardiac glycosides are mainly contained in the leaves, predominantly in the cell vacuoles (LÖFFELHARDT *et al.* 1979; HARKISS *et al.* 1981). *C. distinctus*, which feeds on *C. majalis* leaves by ingesting the contents of their cells, appears to be resistant to these toxic compounds. The effect of this insect on the host plant has many negative aspects: in addition to lowering the biomass of the leaves, it may lower the content of the active substances stored in the subterranean organs (SCHRUTKA-RECHTENSTAMM & LÖFFELHARDT 1985). Moreover, many species of fungi and bacteria can penetrate into a damaged leaf blade more easily and cause diseases such as anthracnose, leaf spot or rust, resulting in chlorosis and deformation of the leaves (MCKEEN & ZIMMER 1964; PIRONE 1978; JAIN *et al.* 2019). Our initial morphological and anatomical analyses of *C. majalis* showed that the epidermis on the upper and lower leaf blade surfaces had the same structure, consisting of thin-walled prosenchymatic cells and a similar number of stomata on both leaf sides. We found that the thickness of the cuticle layer was the same over the entire leaf surface and on the veins on both the upper and lower leaf surfaces. This stands in contrast to e.g. *Mentha* x *piperita*, where the cuticular layer is thicker on the surface of the midrib, thereby presenting a mechanical barrier to thrips feeding (KUCHARCZYK *et al.* 2019). Based on these observations, it can be assumed that the insect feeds on both surfaces to an equal extent, as there were no differences in the epidermal structure on the upper and lower sides of the leaf. Despite the absence of such differentiation in the Lily of the Valley, traces of *C. distinctus* larval feeding were

detected only between the midribs. Our study showed that the vascular bundles, consisting of xylem and phloem elements surrounded by a parenchyma sheath, were also surrounded by a supporting tissue composed of sclerenchymatous fibres. On both sides of the vascular elements, this tissue formed compact layers reaching the upper and lower epidermis, thereby providing a mechanical protection to these elements. It can be assumed that this thick layer of supporting tissue (sclerenchyma) surrounding the veins served as an effective barrier against this insect, and determined the characteristic manner of the *C. distinctus* feeding on the plant. Moreover, the analysis of the tissue structure of *C. majalis* leaves revealed damage to the leaves caused by feeding only in the epidermal layer on both the lower and upper sides of the leaf blade, while no damage to the assimilation parenchyma (mesophyll) was noted. The relatively small superficial damage to the leaf tissue was probably associated with the small size of the weakly chitinised mouthparts of the *C. distinctus* larvae.

In summary, the current report describes the life cycle of *C. distinctus* and the morphology of its preimaginal stages in detail, i.e. an issue that has never been analysed and described before. Importantly, this is a revision of the previous description of its second larval instar, providing a complete picture of its development. Moreover, the research was significantly extended by analysing changes in the morphological and anatomical structure of *C. majalis* leaves caused by the insect feeding, which was described for the first time.

Author contributions

Research concept and design: H.K., D.T.; Collection of data: H.K., M.K.; Data analysis and interpretation: H.K., D.T.; Writing the article: H.K., D.T.; Critical revision of the article: H.K., M.K., D.T.; Final approval of the article: H.K., M.K., D.T.

Conflict of Interest

The authors declare no conflict of interest.

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