

**Morphology of the mature larva and pupa of *Diplapion confluens*
(KIRBY, 1808) (Coleoptera: Apionidae)**

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ABSTRACT. Aim of the work is a detailed description of the mature larva and pupa of *Diplapion confluens* (Kirby). Diagnostic characters of immature stages, information about life-cycle and ecology of this species are presented.

KEY WORDS: Coleoptera, Curculionoidea, Apionidae, Ceratapiini, *Diplapion confluens*, morphology, larva, pupa, life-cycle.

INTRODUCTION

The genus *Diplapion* REITTER, 1916 is mostly distributed over the western part of the Palearctic, and especially the Mediterranean region (MAZUR 2001). A total of seven *Diplapion* species have been recorded in Europe, of which three are found in Poland (WANAT & MOKRZYCKI 2005, ALONSO-ZARAZAGA 2008). Most of them are termophilous or xerothermophilous species, connected with plants from the family *Asteraceae* DUM. Therefore their occurrence is mainly limited to xerothermic plants associations. Most *Diplapion* species are considered as rare (BURAKOWSKI et al. 1992). The taxonomic and systematic position of this genus is defined by ALONSO-ZARAZAGA (1990) and WANAT (1995).

Diplapion confluens (KIRBY) is a Palearctic species. Except of a few countries it is recorded almost from the whole area of Europe. In Poland it is recorded from most regions including also some mountain areas (BURAKOWSKI et al. 1992). It is an eurytopic, xerophilous species, connected with xerothermic and ruderal plant communities or dry meadows

(KOCH 1992). *Matricaria chamomilla* L., *M. discoidea* DC, *Tripleurospermum inodora* (L.) Sch. Bip., *Anthemis arvensis* L. and *A. tinctoria* L. are known as host plants. The biology of *D. confluens* is known, the larva feed inside the roots (SMRECZYŃSKI 1965, BURAKOWSKI et al. 1992, HINZ & MÜLLER-SCHÄRER 2000).

The number of *Apionidae* species, of which immature stages are completely described and illustrated, is relatively sparse (EMDEN 1938, SCHERF 1964, WILLIAMS 1968, ŁĘTOWSKI 1991, MAY 1993).

Only some information about morphology of *Diplapion stolidum* (GERM.) larva is given by van EMDEN (1938). Here, the first detailed, illustrated description of the preimaginal stages of *Diplapion* species is presented.

MATERIAL AND METHODS

25 exemplars of different larval stages (including 9 specimens of mature larvae) and 21 pupae.

The insects were collected in: Kały II ad Zamość (UTM nets: FB 41) and Opoka Duża ad Anopol (EB 63), on May 17th, June 10th, 16th, 20th, 22nd, 30th, July 10th, 15th, 20th 2008.

The specimens were collected on roots of golden chamomile (*Anthemis tinctoria* L.) growing on xerothermic plants communities: *Cariceto-Inuletum* (Kały II) and *Brachypodio-Teucrietum* (Opoka). In order to correctly determine the development stages of *Diplapion confluens*, some larvae were kept alive under laboratory conditions until pupation and then until metamorphosis. The preimaginal stages were preserved in liquid of 75% alcohol. The punctured larvae and pupae were rinsed in distilled water and cleared in 10% solution of potassium hydroxide (KOH) and finally stored in 10% glycerin to prepare microscopic slides. The drawings were made using microscope and camera lucida (MNR-1, 10^X, PZO). The terminology of SCHERF (1964) and MAY (1977, 1994) was used in the morphological description of larva and pupa.

RESULTS

Morphological description

Larva (Figs 1-5)

Body length (mature larva): 1.70 – 2.30 mm (mean 2.10 mm); head width: 0.43 - 0.45 mm (mean 0.44).

Body stout, C-shaped curved, white, head light brown, setae short, light yellowish. Cuticle on the thoracic and abdominal segments minutely spiculate. Body with reduced chaetotaxy (Figs 1–3). Long setae only on thoracic segments, abdomen with microsetae (visible under 400x magnification). Prothorax with 4 long setae (prns) on anterior margin, 2 setae

dorsopleurally (dpls) and a seta ventropleurally (vpls) on each side. Mesothorax with 2 microsetae prodorsally (prs), one micro- and one macroseta postdorsally (pds) and a pair of unequal in length setae dorsopleurally (dpls), on each side. Chaetotaxy of metathorax similar to chaetotaxy on mesothorax. Each pedal area of thoracic segments with 2 macro- and 3-4 microsetae. Each thoracic segments I-III with two microsetae placed ventrally between pedal lobes. Abdominal segments I-VII with 1 microsetae prodorsally (prs), 1 microsetae postdorsally (pds) and 1 microseta dorsolaterally (dls) on each side. Segment VIII posses 2 microsetae postdorsally on each side. Segment IX with 2 very small microsetae dorsally (ds) and one microseta pleurally (ps) on each side. Abdominal segment X reduced to two long, anal lobes about equal size (Figs 1-3). Spiracles (8 pairs) one cameral (Figs 4, 5); first one located between pro- and mesothorax, next ones placed medio-laterally on abdominal segments I - VII.

Head subglobose, frontal suture Y-shaped, poorly visibly, endocarinal line long, extended almost to epistoma (Figs 6, 7). Macro setae of head: 10 dorsal epicranial setae 2 (des_{1-5}). des_1 , placed near of suture coronalis, des_2 located in vicinity of suture frontalis. One microseta placed close to des_2 . des_3 and des_4 localized centrally in each half part of epicranium. des_4 twice shorter than other epicranial setae. des_5 placed near of ocellus. Frontal setae 2 (fs_1 and fs_2) equal length, localized near of epistoma. Lateral epicranial setae les_1 twice longer than les_2 . Post epicranial setae 2 (pes_1 , pes_2) short and blunt. Epicranium with 4 pores on each side, frons with 8 pores. Single ocellus (oc) placed on each side latero-anteriorly. Antenna (Fig. 8) placed near the end of suture frontalis, one-segmented, conical, not exceeding the outline of head; basal membranous area with 2 setal sensille and 2 pores. Clypeus (cl) and labrum (lrm) trapeze-shaped (Fig. 9): clypeus 2.2 times as wide as long with a microseta and a pore localized postero-laterally on each side. Anterior margin of clypeus distinctly sinuate to inside. Labrum about 2.0 times as wide as long with two pairs of labral setae (lrms); median pair (lrm_1) shorter than lateral ones (lrm_2). Anterior margin of labrum slightly sinuate to inside. Epipharynx (Fig. 10) with: 3 pairs of short, straight, median setae (mes_{1-3}), 3 pairs of long, finger like antero-lateral setae (als_{1-3}); and 2 pairs of antero-medial setae ($ams_{1,2}$). ams_1 long, slightly curved placed antero-laterally, ams_2 short, placed medially. Tormae (t) distinctly, elongated, convergent in the anterior parts. Mandible (Fig. 11) broad, bifid, with 1 dorsal seta and a pore on the dorsal area; teeth of unequal height, truncate, molar edge with triangular tooth. Maxilla (Fig. 12) consists of triangle cardo (cd), stipes (st), mala (ma) and maxillary palp (mp). Stipes and mala fused, stipes with a one stipal (stps) and 2 palpiferal ($pfs_{1,2}$) setae and a pore. Mala with 5 slightly curved finger-like setae dorso-apically (dms) and 2 setae ventro-apically (vms); vms smaller than dms. Maxillary palp 2-segmented; segment basal almost equal in length and width, segment distal longer than wide, length ratio of segments basal and distal 1.4 : 1. Segment basal with a microsetae and long, blunt seta laterally, distal with a pore and tiny conical, cuticular processes apically. Praelabium (plb) (Fig. 13) narrow, triangular with a well visible Y-shaped premental sclerite and a pair of setae, next pair of very short setae placed on ligula. Postlabium (pslb) with 3 pairs of unequal in length setae, localized laterally and posterome-

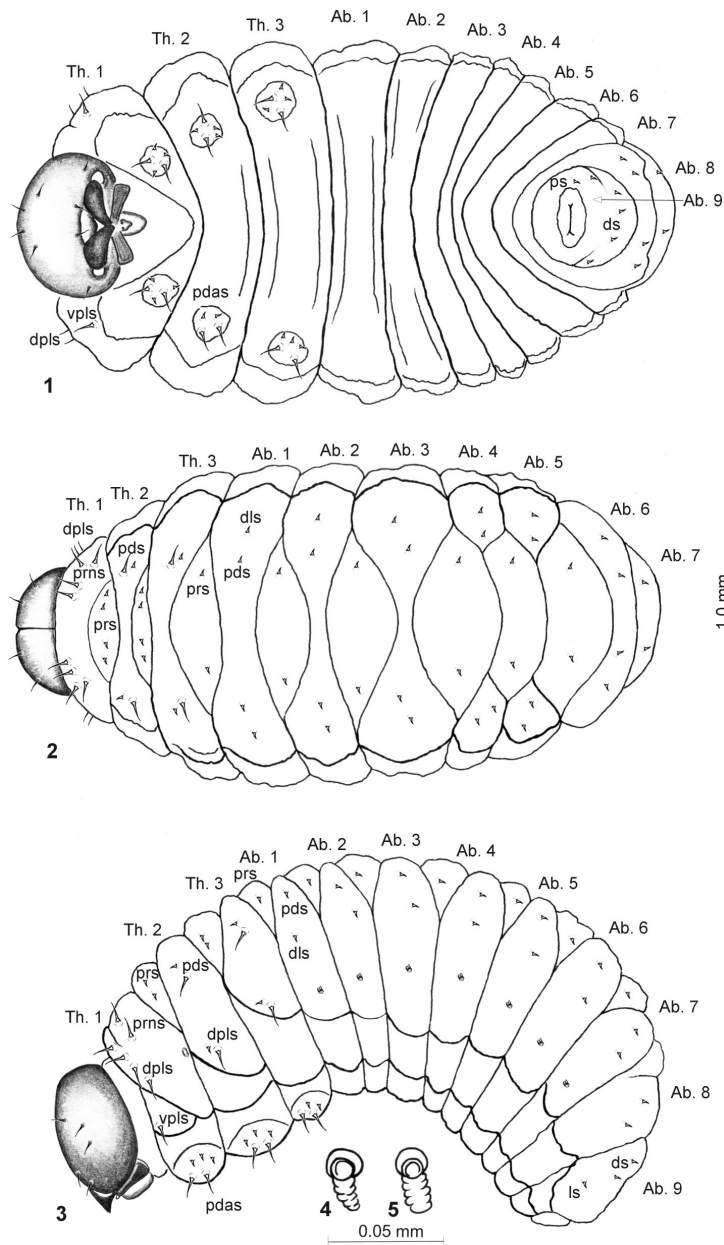
dially. Labial palp (lpa) one-segmented, very small (almost twice long as wide), localized antero-laterally. Each palp with a pore, micro seta and tiny conical, cuticular processes apically.

Pupa (Figs 14-19)

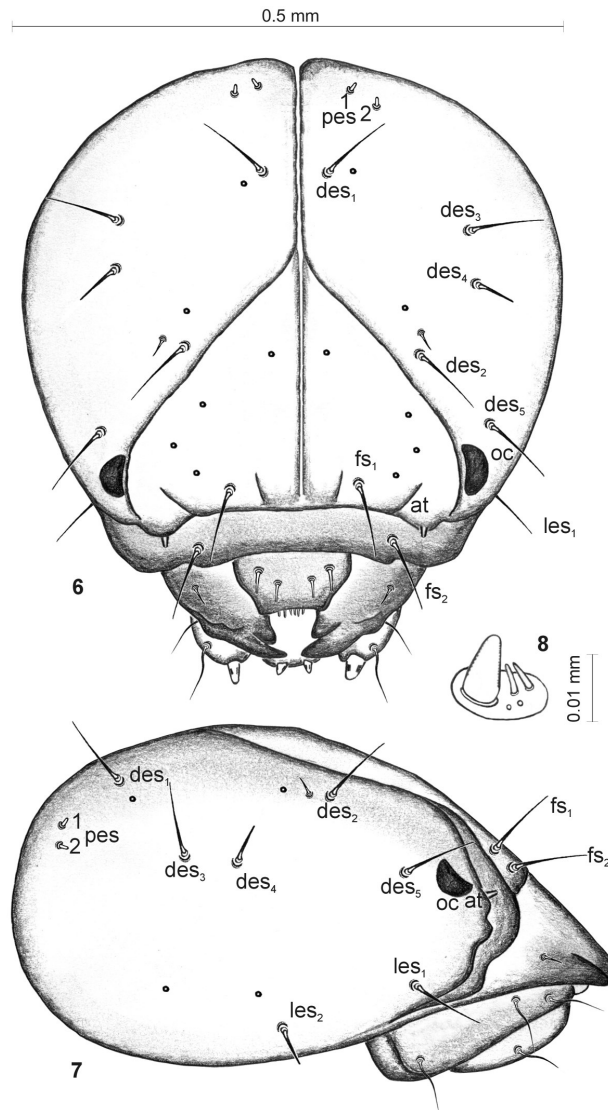
Body length: 2.00 - 2.41 mm (mean 2.11 mm), width: 1.00-1.12 mm (mean 1.10 mm). Body relatively stout, white or grayish-white; setae long, yellow, placed on small protuberances. Head with 2 long, vertical setae (vs), 2 super orbital setae (sos), and a pair of very short, post antennal setae (pas). Rostrum without setae, very long, about 5 times as long as wide, exceed apices of coxae of third pair. Antennae relatively long, capitulum covered by conical cuticular processes. Pronotum 1.5 times as wide as long, at the base with 4 apical setae 2 (as₁, as₂), 2 sublateral setae (sls), 2 lateral setae (ls), a pair of discal setae (ds) and 4 posterolateral setae 2 (pls₁, pls₂) about equal length. Meso- and metanotum almost equal length, each with 2 pairs of long setae, localized medially. Each femora with a long, thin seta placed apically. Abdominal segments I – IV almost equal length, next segments gradually widened and narrowed to the end of body. Pseudocerci (pc) on IX abdominal segment, elongated, narrow, with lens-shaped terminal part, slightly directed to sides (Fig15). Spiracles on abdominal segments I-VI functional, well visible, placed laterally, between tergites and sternites. Each abdominal segments I – VIII with 2 pairs of short, setae, placed dorso-laterally. Segment IX without seta. Sexual dimorphism in structure of VIII and IX sternites well-marked. The gonotheca in female visibly divided, in male single (Figs 18, 19).

Remarks on ecology and biology

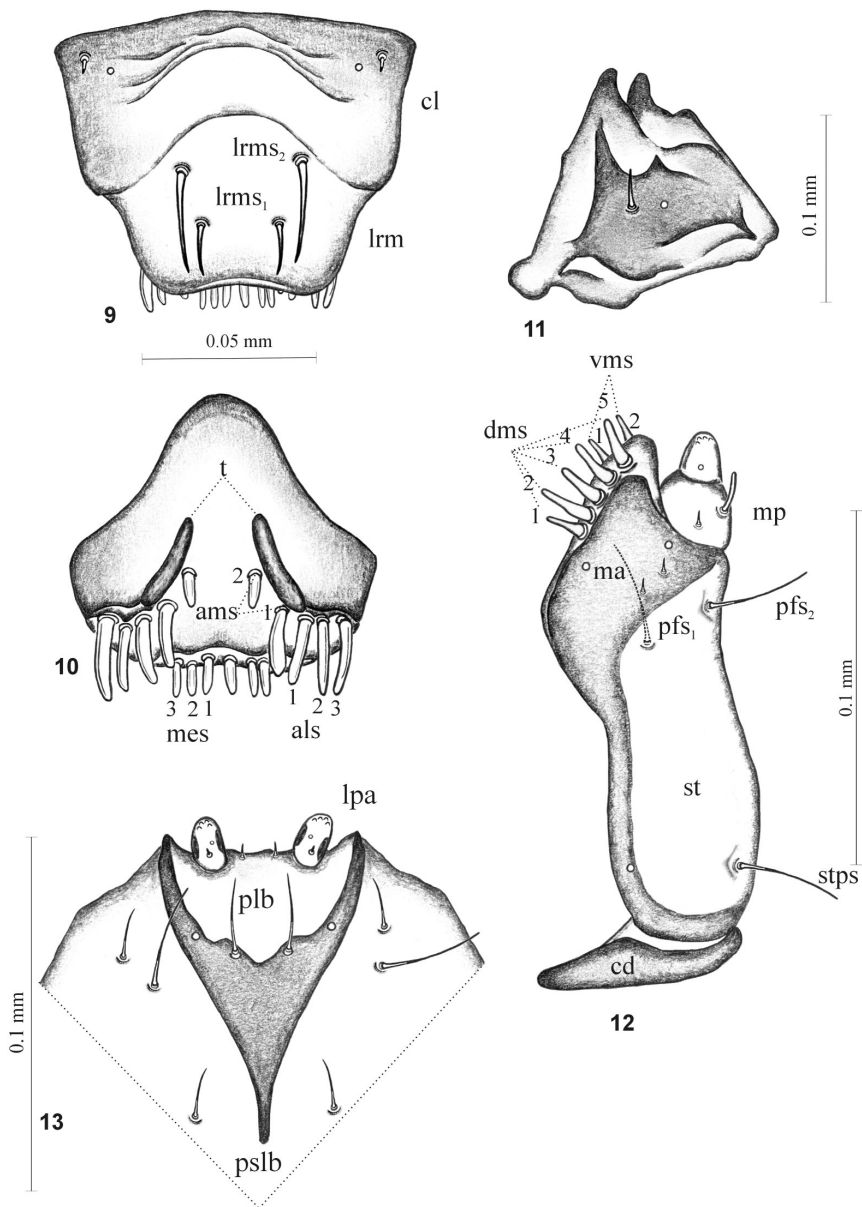
The first active specimens of *D. confluens* were observed in the field as early as in the end of April and the first larval instars were found in middle of May (Fig. 20). Mature larvae and pupae were collected from the end of June to the end of July. The feeding of *D. confluens* larva caused the appearance of a small bulge on the stem. The plants infected by larvae exhibited a decrease in vitality. The development stages of *D. confluens* were observed on up to 10% of specimens of golden chamomile growing at the investigated stations. I found usually 1-5 developing larvae on a single plant. Process of pupation lasts 8-9 days (under laboratory conditions). The sclerotization and dyeing last 3-4 days. Active imagines of the new generation were observed from the end of July to the first decade of October. In the middle of July only a single specimens was observed, whereas after this time a significant increase of abundance was noted. Immature stages infected by parasites or parasitoids were not observed.



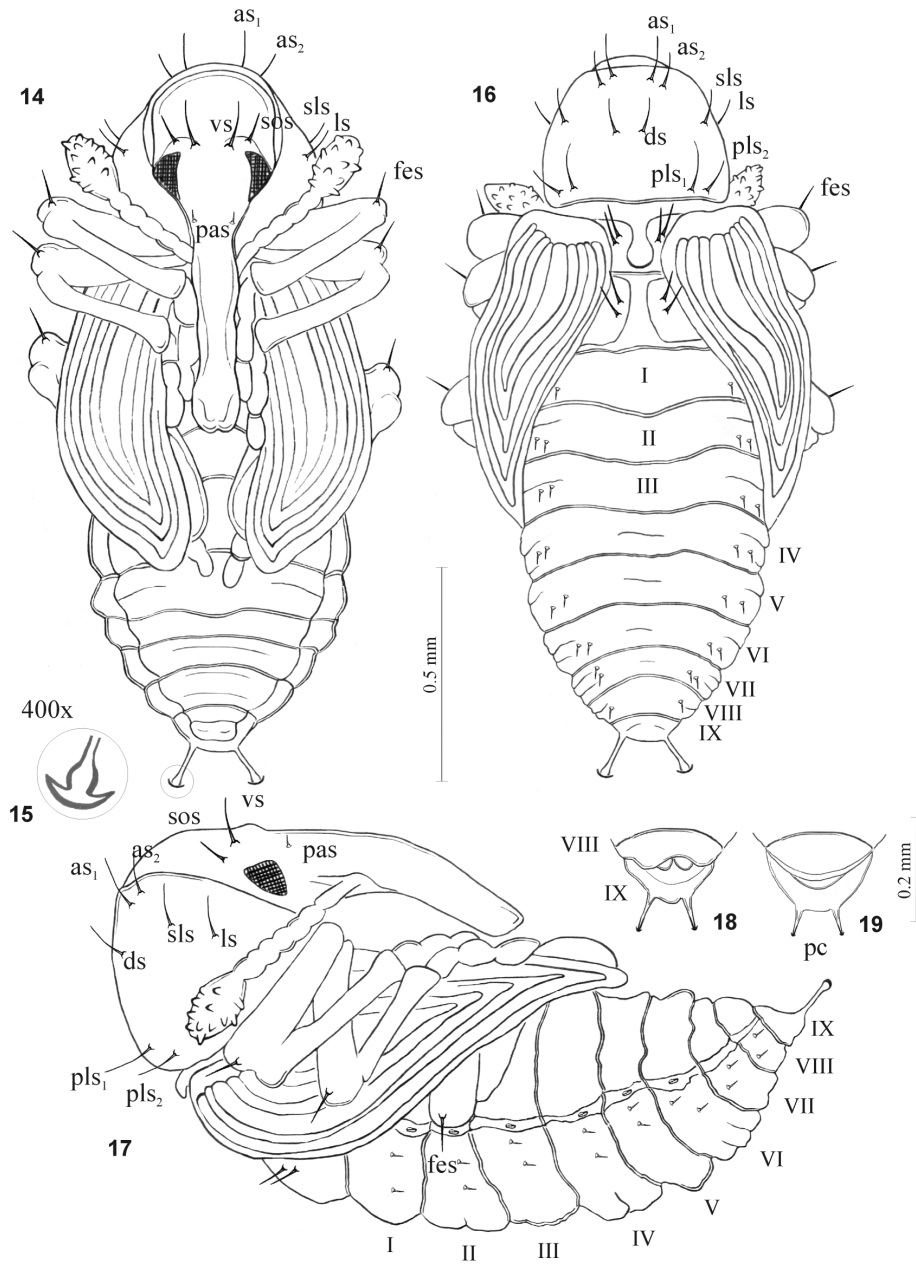
Figs 1-5. *Diplapion confluens*, mature larva, habitus: 1 - ventral view, 2 - dorsal view, 3 – lateral view (prns – pronotal setae, dpls – dorsopleural s., vpls – ventropleural s., pdas – pedal s., prs – prodorsal s., pds – postdorsal s., dls – dorsolateral s., ls – lateral s., ds – dorsal s., Th. 1-3, Ab. 1-9 – number of (Th. – thoracic, Ab.- abdominal) segments, 4 - spiracles of thoracic seg., 5 – spiracles of first abdominal seg.).



Figs 6-8. *Diplapion confluens*, mature larva, head: 6 – dorsal view, 7 – lateral view (des - dorsal epicranial setae, fs - frontal s., les - lateral epicranial s., pes – post epicranial s., oc – ocellus, at - antenna), 8 - antenna.



Figs 9-13. *Diplapion confluens*, mature larva: 9 – labrum (lrm) and clypeus (cl) (lrms – labral setae), 10 – epipharynx (ams - anteromedial setae, als - anteriorlateral s., mes – median s., t - tormae), 11 - left mandible, 12 – right maxilla, dorsal view (dms - dorsally malae setae, vms - ventrally malae s., ma – mala, cd – cardo, st – stipes, mp – maxillary palp, pfs – palpiferal s., stps – stipal s.), 13 - praelabium, ventral aspect (plb – praelabium, lpa - labial palp, pslb – postlabium).



Figs 14-19. *Diplapion confluens*, pupa, habitus. 14 – ventral view (vs - vertical seta, sos - super or bital s., pas – post antennal s., fes – femoral s.), 15 – terminal part of pseudocerci, 16 - dorsal view (as - apical s., ls - lateral s., sls – sublateral s. ds - discal s., pls - posterolateral s.), 17 - lateral view (I - IX – number of seg.), 18 – terminal segment of female, 19 - terminal segment of male (pc – pseudocerci).

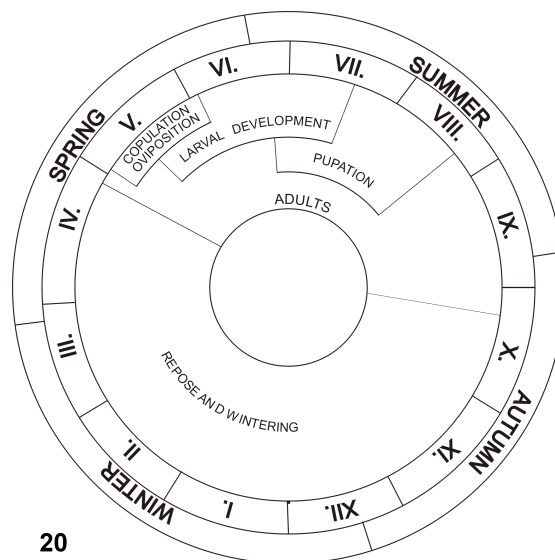


Fig. 20. Life cycle of *Diplapion confluens* under field conditions (I.-XII. – months).

CONCLUSIONS

Diagnostic characters of the mature larva of *D. confluens*: (1) body (mature larva: 0.13 – 0.23 mm), stout, C-shaped; (2) prothorax with 8 prns; (3) spiracles one-cameral; (4) head (width: 0.43 - 0.45 mm) subglobose, endocarinal line present; (5) head with: 10 des, 4 fs, 3 les and 4 pes; (6) antenna one-segmented, conical, basal membranous area with 2 setal sensilles and 2 pores; (7) labrum with two pairs of lrms; (8) epipharynx with: 6 mes, 6 als and 4ams; (9) tormae distinctly, elongated, convergent; (10) molar edge of mandible with triangular tooth; (11) maxilla with 1 stps, 2 pfs; (12) mala with 5 dms and 2 vms; (13) prae-labium triangular with a well visible Y-shaped premental sclerite; (14) labial palp one-segmented.

Diagnostic characters of the pupa of *D. confluens*: (1) body relatively stocky (length: 2.00 - 2.41 mm); (2) head with: 2 vs, 2 sos, and 2 pas; (3) rostrum without setae; (4) antennae relatively long, capitulum covered by conical cuticular processes; (5) pronotum with 4 as, 2 sls, 2 ls, 2 ds and 4 pls; (6) mesonotum with 4 setae; (7) matanotum with 4 setae; (8) each of abdominal segments I – VIII with 4 setae; (9) segment IX without setae; (10) each femora with a single seta; (11) pseudocerci elongated, narrow, with lens-shaped terminal part, slightly directed sideways; (12) spiracles on abdominal segments I-VI placed laterally.

Larvae of only 5 *Ceratapiini* (ALONZO-ZARAZAGA 1990) species have been described so far (EMDEN 1938, SCHERF 1964). In review of European species larvae of: *Omphalapion hookerorum* (KIRBY), *Ceratapion carduorum* (KIRBY), *C. onopordi* (KIRBY), *Diplapion stolidum* (GERM.) and *D. confluens* are described. Some of those species can be differentiated with the following key:

- 1(2)** spiracles unicameral; endocarina very long; anterior margin of labrum slightly sinuate to inside, premental sclerite presents*Diplapion confluens*
2 spiracles bicameral; endocarina short; anterior margin of labrum rounded, premental sclerite absent;
3(4) ocelli two pairs..... *Omphalapion hookerorum*
4 (5) ocelli one pair.....*Ceratapion onopordi*
5(6) ocelli absent.....*C. carduorum*

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