Ultramorphological characteristics of unknown larva of *Phloeonomus punctipennis* Thomson, 1867 (Coleoptera; Staphylinidae; Omaliinae): an obligate saproxylic species: notes on chaetotaxy and ecological preferences

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Abstract

The aim of the study was to describe the morphological ultrastructure, including chaetotaxy, of the previously unknown early (L₁) and late (L₂-₃) larval instars of *Phloeonomus punctipennis*. The diagnostic larval characters for the genus *Phloeonomus* are proposed for the first time. Morphological differences between L₁ and L₃ refer only to the proportion of length of individual parts of antennae and legs as well as length proportion of urogomphi and their subapical setae. The chaetotaxy of the mature larvae of *P. punctipennis* and *Dalotia coriaria*, as a model of aleocharine larvae, is compared. Despite some identified differences, the chaetotaxic system proposed previously for Aleocharinae is successfully applied to the larva of *P. punctipennis*. It may most probably be also useful for larvae of other Omaliinae species. Also, information on geographical distribution and the ecological preferences of this species is provided, as is the information on accompanying insects.

Key words: immature stages, morphology, early and mature larval instar, chaetotaxy, saproxylic species, wood-decay fungus, *Ganoderma applanatum*

Introduction

Saproxylic beetles constitute a very interesting subject of entomological studies. The insects, which are often obligatorily related by scientists to decomposing wood due to their high environmental specificity, belong to the most endangered animals in the world. They constitute an essential element of forest ecosystems by playing a crucial role in maintaining their ecological balance and in protection of biological diversity. They take part in processes that are essential for the correct functioning of the forest, including but not limited to disintegration, decomposition and mineralization of dead wood; they create adequate microenvironments for habitation of various microorganisms (e.g. fungi, bacteria) and they limit the number of other phytophages. Furthermore, saproxylic beetles constitute food for numerous vertebrates, affecting their presence and population numbers. In Central Europe and in Poland, almost 1500 and 1300 beetle species respectively belonging to more than 70 families have been recorded that are connected with dead wood (Gutowski 2006). Among them, one of the most numerous and most diverse families of Coleoptera—in terms of form and ecological specialization—is the Staphylinidae.

The relationship between Staphylinidae and decaying wood is most often not limited to the presence of adult individuals in this special microhabitat—rove beetles also reproduce and develop here. In this context, particular attention should be paid to larval forms, which, being intensively feeding development stadia that do not leave their habitats during the entire development, are of probably higher ecological importance than adult forms. In contrast to imagines, which are relatively well-studied and typically easy to identify by experts, the degree to which saproxylic Staphylinidae larvae have been researched is poor and most often limited to single species representing various sub-families (Staniec 2003, 2004; Pietrykowska-Tudruj, Staniec 2006; Jałoszyński, Kilian 2012; Jałoszyński 2015). Some data contained in papers published so far are either incomplete or obsolete, while brief
descriptions of morphological structures are accompanied by only schematic figures (Paulian 1941; Pototskaya 1967; Kasule 1968; Steel 1970; Topp 1978; Burakowski, Newton 1992). The information given in the referenced works on larval forms is not very helpful with respect to contemporary research e.g. in the scope of phylogenetics, functional morphology or taxonomy. The study subject therefore requires complete and more detailed analyses, offering e.g., ultrastructures or chaetotaxy of various body parts, particularly for close relationship between examined taxa.

Similarly, the representatives of the genus *Phloeonomus* Heer, 1839 have been, thus far, insufficiently studied in terms of external morphology of their larval stages. Pototskaya (1967) includes this genus in the key to identification of Staphylinidae larvae of the European part of Russia. In the key, however, the author only provides very schematic and not very detailed figures of *P. pusillus* larva cited as per Verhoeff (1919) that illustrate elements of the epicranium (epipharynx, antenna, mandible, prementum and maxilla) and the two last abdominal segments. Steel (1970) in his paper on Staphylinidae larvae from the subfamily Omaliinae of British fauna, provides a drawing that presents the two terminal, abdominal segments of a larva of the mentioned species. In the same paper, the author presents illustrations of some morphological structures with concise descriptions of mature larvae representing a total of 30 genera of Omaliinae, including 23 British and 7 non-British. However, the data provided in the paper are scarce and not very detailed and, besides, do not cover many important external structures, including the chaetotaxy which is important in phylogenetic studies.

The genus *Phloeonomus* includes a total of 57 species of which 10 have been demonstrated to exist in the Palearctic, while only 4 in Central Europe and Poland. These are very small beetles (body length of European species ranges from 1.2 to 2.0 mm), dorsoventrally flattened, with a matt and slightly dotted body surface (Fig. 40) (Koch 1989; Herman 2001; Zanetti 2012; Melke 2014). All European species are related to dead wood and most often inhabit the polypores located on the trunk or under the bark.

The current paper presents a detailed description of the external structure of a previously unknown *P. punctipennis* larva including its chaetotaxy and morphological differences between the early (L₁) and late (L₂–₃) larval instar. Also, information on geographical distribution and the ecological preferences of this species is provided, as is the information on accompanying insects.

**Materials and methods**

**Experimental material.** A sporocarp of *Ganoderma applanatum* (Pers. Ex Wallr.) inhabited by various developmental stages of *P. punctipennis* was collected at one site in south-eastern Poland: Dzbeniński Forests near Lublin (SE Poland), 51°17ˈ01.12"N, 22°30ˈ46.08"E, 242 ASL. The sporocarp inhabited by insects was collected from a fallen trunk of *Quercus* sp. on 17th November 2015 in a suburban, highly-shaded, mid-field mixed (mostly hornbeam) deciduous forest covering an area of approx. 9 ha. The forest stand comprised several dozens of 100–200 years old oak trees.

All specimens of insects were removed from the polypore, killed with boiling water and preserved in ethanol (75%). In total, 12 larvae were selected from the sporocarp which represented: the first (L₁)—1 ex., second (L₂) —2 exx and third (L₃)—9 exx larval instars, as well as two adult individuals of *P. punctipennis* and a few adults of minute tree – fungus beetle—*Cis castaneus* (Herbst, 1793) (Coleoptera, Ciidae). The preserved material of *P. punctipennis* was used in further analysis.

**Study techniques.** The specimens were measured using an Olympus BX63 compound microscope. Measurements were made in cellSens Dimension v. 1.9 software and given in millimeters. For preparation of morphological analyses, the preserved larvae were treated with a 10% KOH solution for approximately twelve hours, rinsed in distilled water, and placed in lactic acid for subsequent preparation and placement of mouthparts and sensory structures on temporary microscope slides. Photographs showing total aspects of the adult, larvae (L₁, L₃) of *P. punctipennis* and adults of *C. castaneus* were taken with an Olympus DP72 digital camera mounted on an Olympus SZX16 compound microscope (Figs 1–3, 40–41), an Olympus DP21 digital camera mounted on an Olympus BX63 compound microscope (Figs 13, 16–19, 21, 21c, 22, 26, 28, 36–37) or with a VEGA3 TESCAN SEM (Figs 4–12, 14–15, 17a, 18a, 19a–a1, 20, 20a–b, 21a–b, 23–25, 27, 27a, 29–35, 38–39a), and subsequently corrected using CorelDRAW Graphics Suite X6. The material examined for morphological measurements is shown in Table 1.
Generic diagnosis of the mature larvae

The diagnostic characteristics of the *Phloeonomus* genus are determined based on morphological data of the following species: *P. pusillus* (Gravenhorst, 1806) and *P. punctipennis* (Pototskaya 1967; Steel 1970; the present study). The combination of characteristics that allow to distinguish the mature larvae of *Phloeonomus* from known larvae of other genera within the subfamily Omaliinae: (1) dorsal ecdysial lines bifurcate on the setae Ed2–3 level (Figs 9, 10); (2) different-sized ocelli, the two lowest contacting one another (Fig. 10a); (3) sensory appendage of antennal article II slightly curved inside, equal in length to article III (Figs 13, 15); (4) labrum trapezoidal; (5) anterior margin of labrum, between setae Lm1 and Ld1 slightly convex (Figs 17, 17a); (6) mandibles with very well developed membranous prostheca (Figs 19, 19a); (7) mandibles with row of saw-like teeth along inside edge dorsally (Figs 20, 20b); (8) right mandible with one, left mandible with two subapical teeth (Figs 19, 20, 20a); (9) adoral margin of mala with bifurcate tooth (Figs 21, 21b, 21c); (10) prementum very broad (Fig. 26); (11) ligula broad and rounded; (12) urogomphi slightly curved upwards (Fig. 35); (13) urogomphi and abdominal segment X almost equal in length; (14) at least some long setae on abdomen bifurcate near top (Figs 4, 5).

Morphological description of larval stage of *P. punctipennis*

Third (last) larval instar (L₃) (Figs 1–3). Body semi cylindrical, moderately dorso-ventrally flattened, head and prothorax almost uniform width, thoracal segments gradually widened to metathorax; first abdominal segments somewhat narrower than second; segments II–VI almost uniform width and then abdomen narrowed to terminal segment of body. Colour: head light brown, sufficient strongly sclerotized with darker ocellus and mouth parts; tergite of prothorax, abdominal segment VII, VIII, anterior regions of other tergites of thorax and abdominal tergites I–VI yellow; terminal segment IX and X dark brown, strongly sclerotized with almost black urogomphi; sternites almost colorless; bases of all tergites (except of segment X) with transverse carina anteriorly. All setae light brown; setae on head and sternites simple (Fig. 6), some long setae on thoracal and abdominal tergites bifurcate (Figs 4, 5). Microstructure of tergites reticulate, well visible on head and abdominal segment IX–X (Figs 7, 8, 35a).

Head (Figs 9–12): as long as wide, widest at level of setae Em1, side margins distinctly rounded; dorsal ecdysial lines (Es) bifurcate in one fifth length of the head from the base (at level of setae Ed2–3); each side of head with 5 oval, weekly convex, brown ocellus (Oc) (Figs 10, 10a), Chaetotaxy of dorsal side with 32 setae—18 frontal [2(Fl1–4, Fd1–3, Fm1–2)], 8 epicranial [2(Ell, Ed1–3)] and 6 posterior micro setae (2P1–3), and 4 pores 2(Fc1, Ec1) (Fig. 9). Lateral side with 12 setae [2(Em1–2, T1, L1–3)] (Fig. 10). Ventral side with 8 setae 2(V1–3, V1). Functional position of antennae (At), labrum (Lr), epipharynx (Ep), mandibles (Md), maxillae (Mx), hypopharynx (Hp) and labium (Lb) as in Figs 11–12.

Antenna (Figs 13–15): three articulated, length ratio of articles I–III: 1.2:2.2:1, respectively. Article I almost 1.4 ‘as long as wide, with 4 pores; article II 1.2 ‘as long as wide, with 3 macro setae, one finger-shaped sensory appendage (Sa), slightly curved upside and slightly narrowed to apex, and 3 solenidia ventrally of different size (IIS1–3) (Fig. 15); article III 2 ‘as long as wide, with 3 macro setae and 4 solenidia apically (IIS1–4) of different length. Labrum (Figs 17, 17a): trapeziform in outline, narrowed anteriorly, 2.1 ‘wider at the base than anterior margin; slightly sinuate anteriorly with central part somewhat convex (Fig. 17a); with 4 macro (2Ld2, 2Lm1) and 6 micro [2(Ld1, La1, Lv1)] setae; separated from clypeal region by transverse line. Adoral surface of labrum (epipharynx) (Figs 18, 18a): membranous with 4 (Acp, Mcp1–2, Pcp) transversal rows of cuticular processes directed to pharynx (Ph) broken in half of their length; central area with a pair of fore and 8 hind pores. Mandibles (Md): moderately elongated, less acute apically, with 2 macro setae about outer margin and 2 pores, widened basally; incisor lobe with one of subapical tooth (T1) in right of Md (Fig. 19) or two of subapical teeth (T1–2) in left of Md (Figs 20, 20a) and, in both Md, with row of about 30 saw-like teeth along inside edge dorsally (Figs 20, 20b),...
20b); molar lobe with very developed membranous prostheca (Pst) (Figs 19, 19a); prostheca with thick, brush-like cuticular processes forming about 40 transversal rows (Fig. 19a1). Maxilla (Mx) (Figs 21, 22); consisting of triangular cardo (Cd) divided by sclerotized ridge into two unequal parts, shorted stipes (Stp), elongated and almost uniform width mala (Ma) slightly obliquely truncate and three articulated maxillary palp (Pm) (Fig. 21); cardo with 1 ventral setae; stipes with 3 setae (1 ventral and 2 lateral); palpifer (Pf) with 1 seta and one pore; mala weakly separated from stipes by unclearly visible line, with 2 setae (1 ventral and 1 lateral) and 1 pore; adoral margin of mala (functional positions in Fig. 12) with 2 rows of 6–8 teeth different size and shape (Figs 21, 21c), the most proximal tooth bifurcate (Bt) (Fig. 21b); inner margin (under bifurcate tooth) of mala with row of a few (usually 6–7) spinose cuticular processes. Maxillary palp (Pm) (Fig. 21): length ratio of articles I–III: 1.3:1:1.8, respectively; article I wider than second, 1.8 ’ as long as wide with 2 pores, article II 1.9 ’ as long as wide with 2 setae, article III narrower than I and II, 5.9 ’ as long as wide, with 1 digitiform sensory appendage basally about half length of article, 1 pore and 4 tiny sensory appendages apically (Fig. 21a). Hypopharynx (Figs 23–25): membranous, surface densely pubescent of equal in length triangular cuticular processes, forming transversal rows; sides with smaller triangular cuticular processes (Cp) grouped in lobes laterally (Fig. 23); apex with 3 pairs of button like sensilla (S1–3) (Fig. 25). Labium (Fig. 26): ligula (Lg) broad and rounded; prementum (Pmnt) trapeziform, strongly transverse well sclerotized with 2 macro setae and a few of triangular cuticular processes lateroanteriorly; mentum (Mnt) membranous in anterior part, with 4 macro setae and a pair of pores; submentum (Smnt) with a pair of macro setae; labial palps slightly longer than ligula, two articulated almost equal in length, article I with 1 pore laterally, article II distinctly narrower than first, with 1 pore ventrally and 6 small sensory processes apically (Fig. 25).

Thorax. Foreleg (Fig. 27): consist of stocky coxa (Cx), short trochanter (Tr), moderately elongated femur (Fe) twice as long as wide, slim tibia (Tb) 4.2 ’ as long as wide and slightly curved inwards tarsungulus (Ts) 4.1 ’ as long as wide; Fe with 8 setae (Ad1, Av1–2, A11, D1, Pd1, Pvi1–2); Tb with 9 spine-shape setae (Ad1–3, Av1–2, Pd1–2, P11, Pvi1) and a few spines anteriorly; Ts with 2 spine-shaped setae (Fig. 27a). Length ratio of Fe, Tb and Ts: 1.9:2.2:1, respectively. Length ratio of pronotum (Pnt), mesonotum (Msn) and metanotum (Mnt): 1.3:1:1.1, respectively. Pnt with 36 setae [2(A1–5, L1–3, Da1–2, Db1–2, Dc2, Dd1, P1–4)] and 12 pores [2(C1–6)] (Fig. 29); Msn with 32 setae [2(A1–5, L1–2, Da2, Db1–2, Dc2, Dd1, P1–4)] and 6 pores [2(C2, C4, C6)]; chaetotaxy of metanotum identical to mesonotum; lateral area between pro- and mesothorax with a pair of functional spiracles (Sp) (Fig. 29a), and between meso- and metathorax with a pair of atrophied spiracles (Asp) and 1 micro seta; pleurites (Pl) of meso- and metathorax residual each with 1 seta. Prosternum (Fig. 30): with 16 setae [2(Eu1–2, Pr1–2, St1–4)].

Abdomen. Chaetotaxy of tergites: I–VI (Fig. 31) with 24 setae [2(A2, A3, A5, Da2, Db2, Dc2, L1, L3, P1–4)] and 4 pores [2(C1, C2)]; VII and VIII (Fig. 32) each with 26 setae [2(A3, A5, Da2, Db2, Dc2, L1, L2, P1–6)]; IX (Fig. 35) with 8 setae [2(A2, Dc2, L2, P6)] and 2 pores [2(A2)]. Chaetotaxy of sternites: I (Fig. 33) with 8 setae [2(D1, P1–3)]; II–VI (Fig. 33) with 12 setae [2(Ps1, D1–2, P1–3)]; VII and VIII (Figs 32, 34) with 16 setae [2(Ps1, D1–2, P1–5)]; IX (Figs 35, 38) with 10 setae [2(Ps1, D2, P1–3)]; reticulate microstructure as in Fig. 35a. Abdominal segments I–VI each with a pair of paratergites (Pte) and parasternites (Pst) laterally; each parategites with 2 setae, parasternites of segment I each with 1 setae, others each with 2 setae (Fig. 31). Segment X narrow, cannulaten (Figs 35, 38); tergite and sternite fused in uniform rings with 8 setae [2(coded: 1–4)] and 4 anal vesicles apically (Av) covering by rows of triangular hooks (Figs 38, 39, 39a). Urogomphi (Ug) (Figs 35, 37, 38): one-articled, moderately elongated, straight, gradually narrowed to apex, 3.5 ’ as long as wide at the base; with 8 setae (4 long, 4 short; coded:1–8), among them 2 (1 long, 1 short) subapical (Fig. 35b); length ratio of Ug and subapical long seta: 1.1–1.3:1, respectively; Ug and segment X (pygopod) about equal in length (Figs 35, 37, 38).

First larval instar (L1) (Figs 16, 28, 36). The main differences between L1 and L2–L3 of P. punctipennis involve:

1) structure of antenna—a) length relation of article I–III, in L1—1:1.9:1.3, in L2–L3—1:2:2.2:1, b) length to width ratio of article I and II, in L1—1:1.5 and 1.8:1, respectively, in L2–L3—1:4:1 and 2.5:1, respectively, c) length to width ratio of sensory appendage (Sa) of article III, in in L1—5:1, in L2–L3—3.5:1 (Figs 13, 16); (2) structure of legs—a) length to width relation of tibia, in L1—short and stocky (Fig. 28), 2.7 ’ as long as wide, in L2–L3—elongated, slim (Fig. 27), 4.2 ’ as long as wide, b) length ratio of Fe, Tb and Ts, in L1—1:1.4:1, in L2–L3—1.9:2.2:1; (3) length relation of urogomphus and long subapical seta, in L1—1:1.4 (Fig. 36), in L2–L3—1:2:1 (Fig. 37).

Some measurements of all larval instars are shown in Table 1.
FIGURES 1–12. Phloeonomus punctipennis, mature larva. 1–3, entire dorsal (1), lateral (2) and ventral (3) aspect; 4, 5, bifurcate setae of abdominal tergites; 6, simple seta of head; 7, 8, microstructure of head of posterior part (7) and around of frontal suture (8); 9–12 head in dorsal (9), lateral (10) with arrangement of ocelli (10a) and posterior setae (10b), and frontal aspect (11, 12). Abbreviations: At, antenna; Bt, bifurcate tooth; Ec, epicranial campaniform sensilla; Ed, epicranial dorsal setae; El, epicranial lateral setae; Em, epicranial marginal setae; Ep, epipharynx; Es, epicranial suture; Fd, frontal dorsal setae; Fl, frontal lateral setae; Fm, frontal marginal setae; Fr, frons; Hp, hypopharynx; L, lateral setae; Lb, labiunm; Lr, labrum; Lp, labial palp; Ma, mala; Mdr, mandible right; Mdl, mandible left; Mx, maxilla; Mnt, mentum; Mp, maxillary palp; Oc, ocellus; P, posterior setae; Pst, prostheca; T, temporal setae; V, ventral lateral setae.
FIGURES 13–18. Phloeonomus punctipennis, larva. First larval instar (16), third larval instar (13–15; 17–18). 13–16, right antenna in dorsal aspect (13, 16) and in ventral aspect (15), antennal article III (14); 17, labium and anterior margin (17a); 18, epipharynx and anterocentral area (18a). Abbreviations: I–III, antennal articles; IIS, solenidia of antennal article II; IIIS, solenidia of antennal article III; Acp, anterior row of cuticular processes; Bt, bifurcate tooth; La, labral anterior setae; Ld, labral dorsal setae; Lm, labral marginal setae; Lv, labral ventral setae; Mep1, -2, middle rows of cuticular processes; Pcp, posterior row of cuticular processes; Sp, spores.
LARVAL INSTARS OF *PHLOEONOMUS PUNCTIPENNIS*

**FIGURES 19–22.** Phloeonomus punctipennis, larva. 19–20, right mandible in ventral aspect (19) and prostheca (19a, 19a1); left mandible in ventral aspect with portion around apex (20a) and internal ridge (molar lobe) in dorsal aspect (20b); 21, anterior portion of right maxilla with apex of maxillary palp (21a), characteristic bifurcate tooth on adoral margin of mala (21b) and apical portion (21c) in ventral aspect; 22, left maxilla in ventral aspect. Abbreviations: I–III, articles of maxillary palp; Bt, bifurcate tooth; Cd, cardo; Ma, mala; Pf, palpifer; Pm, maxillary palp; Pst, prostheca; Stp, stipes; T, subapical teeth of mandible.
FIGURES 23–28. *Phloeonomus punctipennis*, larva. First larval instar (28), third larval instar (23–27). 23, 24, hypopharynx in dorsal (23) and frontal (24) aspect; 25, apex of ligula and labial palps; 26, labium; 27, 28, foreleg in anterior aspect and tarsungulus (27a). Abbreviations: I, II, articles of labial palp; A, anterior setae; Ad, anterodorsal setae; Al, anterolateral setae; Av, anteroventral setae; Cp, cuticular processes; Cx, coxa; Fe, femur; D, dorsal setae; Lg, ligula; Mnt, mentum; Pd, posterodorsal setae; Pmnt, prementum; Pl, labial palp; Pv, posteroventral setae; S1–3, apical sensillae of ligula; Smnt, submentum; Tb, tibia; Tr, trochanter; Ts, tarsungulus.
FIGURES 29–34. *Phloeonomus punctipennis*, mature larva. 29, thorax in lateral aspect; 30, prosternum; 31, abdominal segments I and II in lateral aspect; 32, abdominal segments VII and VIII in lateral aspect; 33, abdominal sternites I and II; 34, abdominal sternite VIII.Abbreviations: I–II, VII–IX, abdominal segments or sternites; A, anterior setae; Asp, atrophied spiracle; C, campaniform sensilla; Cx, coxa; D, Db, Dc, discal setae, rows a–c; Eu, eusternum; Hd, head; L, lateral setae; Msn, mesonotum; Mtn, metanotum; P, posterior setae; Pl, pleurite; Pnt, pronotum; Pr, presternum; Pte, paratergite; Ps, pre sternal setae; Pst, parasternite; Sp, spiracle, St, sternellum; Ste, sternite; Te, tergite.
FIGURES 35–41. Phloeonomus punctipennis (35–40) and co-occurring insects (41). First larval instar (36), mature larva (35, 37–39). 35, urogomphus with abdominal segments VIII–X in lateral aspect; 36, 37, urogomphus with abdominal segments IX and X in dorsal aspect; 38, abdominal segments of IX and X in ventral aspect; 39, ventral lobes of anal vesicle with microstructure (39a); adult (40); 41, adults of Cis castaneus Herbst, 1793. Abbreviations: A, anterior setae; Av, anal vesicle; D, Db, Dc, discal setae; L, lateral setae; P, postarior setae; Ps, presternal setae; Ug, urogomphus.
Larval instars of *Phloeonomus punctipennis*

### TABLE 1. Some measurements of all larval instars of *Phloeonomus punctipennis*; A, average; L₁/₁-3/X, larval instars/number of specimens examined; R, range; SV, standard variation. Measurements expressed in millimeters (mm).

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<th>Measurement</th>
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### Notes on distribution, ecological preferences and co-occurring fauna

*P. punctipennis* is known to be present in many European countries and North Africa (Madeira, Azores) (Herman 2001; Löbl & Smetana 2004). This species has been discovered across all of Poland, although somewhat less often than the very similar *P. pusillus*. On the sites of its presence, it was typically observed in several dozens of individuals throughout the entire vegetation season (Melke, unpublished data). The beetle, referred to as an eurytope, silvicol and corticol, i.e., a forest-based species related mainly to deciduous trees (e.g. *Quercus*, *Fagus*, *Betula*), is classified as an obligate saproxylic species. Adults and larvae live under loosened bark and in the polypores that grow on it (Burakowski et al. 1979; Koch 1989; Hagvar & Økland 1997; Kula et al. 1999; Alexander 2002; Schigel 2011).

Due to feeding preferences, the beetle is considered a fungal feeder preferring the fructifications of wood fungi or saproxylophagous species more or less associated with dead wood during the process of its decomposition (Alexander & Anderson 2012; Carpaneto et al. 2015; Sawoniewicz 2013, 2015).

The recent research conducted on the groups of saproxylic beetles in various regions of Poland yielded several new facts regarding the environmental preference of the studied species. And thus, in the Polish lowland region, *P. punctipennis* is clearly related to mixed (mostly hornbeam) deciduous forests, replacing *P. pusillus* that is sporadically recorded in those groups. In turn, in coniferous forests, the proportion between these two species is exactly the opposite. In mountainous and sub-mountainous regions (e.g. Przemyśl Foothills, Bieszczady Mountains), *P. punctipennis* can be found relatively often under fir bark, either alone or together with *P. pusillus*. In the Carpathian Forest it is observed under the bark of fir trees when their branches become infested by fir weevil (*Pissodes piceae*) (Illiger, 1807), sometimes together with species from the *Cerylon* Latreille, 1802 (*Cerylonidae*) and *Placusa* Erichson, 1837 (*Staphylinidae*) genera. In other locations, it was also observed in subcortical environments, simultaneously with the first larval instar of *Cucujus cinnaberinus* (Scopoli, 1763) (*Cucujidae*)—however, these have always been microhabitats with firmly attached bark and high humidity (Melke, unpublished data).

Various development stages (two specimens of adults, one L₁, two L₂, and nine L₃) of *P. punctipennis* were collected from a sporocarp of *Ganoderma applanatum*. The sporocarp was three years old (based on the number of hymenophore layers), dead and rotten to a large extent (approx. 50%). Its condition suggested second degree of decay based on the four-degree scale applied to a different species of perennial polypore (Nadvornaya & Nadvorny 1991).

Together with the studied staphylinids, the sporocarp was inhabited also by various development stages of *Cis castaneus*, a beetle from the family Ciidae that is abundant in Europe (Fig. 41). Both the adults and larvae of this minute tree-fungus beetle feed in living sporocarps of macrofungi. Their development in a single polypore typically involved several generations and the insects of the last generation successfully develop on the remains of a dead sporocarp. The mentioned biological features of this species indicate that it may be considered both a parasite of fungi and a saproophage feeding on decaying polypore sporocarps (Lopes-Andrade 2011). When the material was collected, *C. castaneus* had been present in large numbers as the first larval instar; the number of individuals in later larval and pupal stadia was considerably smaller. The larvae penetrated all parts of the fungus,

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**Notes on distribution, ecological preferences and co-occurring fauna**

*P. punctipennis* is known to be present in many European countries and North Africa (Madeira, Azores) (Herman 2001; Löbl & Smetana 2004). This species has been discovered across all of Poland, although somewhat less often than the very similar *P. pusillus*. On the sites of its presence, it was typically observed in several dozens of individuals throughout the entire vegetation season (Melke, unpublished data). The beetle, referred to as an eurytope, silvicol and corticol, i.e., a forest-based species related mainly to deciduous trees (e.g. *Quercus*, *Fagus*, *Betula*), is classified as an obligate saproxylic species. Adults and larvae live under loosened bark and in the polypores that grow on it (Burakowski et al. 1979; Koch 1989; Hagvar & Økland 1997; Kula et al. 1999; Alexander 2002; Schigel 2011).

Due to feeding preferences, the beetle is considered a fungal feeder preferring the fructifications of wood fungi or saproxylophagous species more or less associated with dead wood during the process of its decomposition (Alexander & Anderson 2012; Carpaneto et al. 2015; Sawoniewicz 2013, 2015).

The recent research conducted on the groups of saproxylic beetles in various regions of Poland yielded several new facts regarding the environmental preference of the studied species. And thus, in the Polish lowland region, *P. punctipennis* is clearly related to mixed (mostly hornbeam) deciduous forests, replacing *P. pusillus* that is sporadically recorded in those groups. In turn, in coniferous forests, the proportion between these two species is exactly the opposite. In mountainous and sub-mountainous regions (e.g. Przemyśl Foothills, Bieszczady Mountains), *P. punctipennis* can be found relatively often under fir bark, either alone or together with *P. pusillus*. In the Carpathian Forest it is observed under the bark of fir trees when their branches become infested by fir weevil (*Pissodes piceae*) (Illiger, 1807), sometimes together with species from the *Cerylon* Latreille, 1802 (*Cerylonidae*) and *Placusa* Erichson, 1837 (*Staphylinidae*) genera. In other locations, it was also observed in subcortical environments, simultaneously with the first larval instar of *Cucujus cinnaberinus* (Scopoli, 1763) (*Cucujidae*)—however, these have always been microhabitats with firmly attached bark and high humidity (Melke, unpublished data).

Various development stages (two specimens of adults, one L₁, two L₂, and nine L₃) of *P. punctipennis* were collected from a sporocarp of *Ganoderma applanatum*. The sporocarp was three years old (based on the number of hymenophore layers), dead and rotten to a large extent (approx. 50%). Its condition suggested second degree of decay based on the four-degree scale applied to a different species of perennial polypore (Nadvornaya & Nadvorny 1991).

Together with the studied staphylinids, the sporocarp was inhabited also by various development stages of *Cis castaneus*, a beetle from the family Ciidae that is abundant in Europe (Fig. 41). Both the adults and larvae of this minute tree-fungus beetle feed in living sporocarps of macrofungi. Their development in a single polypore typically involved several generations and the insects of the last generation successfully develop on the remains of a dead sporocarp. The mentioned biological features of this species indicate that it may be considered both a parasite of fungi and a saprophage feeding on decaying polypore sporocarps (Lopes-Andrade 2011). When the material was collected, *C. castaneus* had been present in large numbers as the first larval instar; the number of individuals in later larval and pupal stadia was considerably smaller. The larvae penetrated all parts of the fungus,
however most often they could be found between the individual layers of the hymenophore, boring horizontal tunnels pointing in various directions. The initial larval instars were observed in the hymenophore tissue, inside the tubes. The adult individuals constituting approx. 15% of the population inhabited mainly the rotten, soft tissue above the hymenophore. Their presence was identified by relatively numerous, almost round exit holes bored by individuals of the new generation. The holes led to tunnels which were used by *P. punctipennis* larvae as shelter. Some larvae move from the tunnels to the surface of the polypore.

**Discussion**

This paper is the first to present a detailed morphological description of a larva belonging to the subfamily Omaliinae, with encoded chaetotaxy the system and terminology of which is referenced as per Ashe & Watrous (1984). Although the mentioned authors used the chaetotaxy of the larva of *Dalotia coriaria* (Aleocharinae) as a model of aleocharine larvae, they did not exclude the possibility of using some aspects of this pattern to other staphylinoid larvae. Our study confirms this view. As it turned out, the system proposed earlier may be, with some modifications, accommodated to develop the chaetotaxy of the *P. punctipennis* larva. With regard to both staphylinoid species listed above, the identical chaetotaxy is present on the femur, tibia and on the ventral side of the head (Tab. 2). Many homologous setae were also found on the dorsal side of the head, pronotum and mesonotum. Proportionally to the number of all setae, most differences in the chaetotaxy of the larvae of both taxa were discovered on the prosternum as well as tergites and sternites of the abdomen. They most often refer to the lack of a row of setae on these body parts in the *P. punctipennis* larva the chaetotaxy of which, as compared to the *D. coriaria* larva, is clearly less abundant. In turn, only few setae present on the head, mesonotum, prosternum and abdominal tergites of *P. punctipennis* do not have their counterparts in *D. coriaria* (Tab. 2). Despite those identified differences, the model of chaetotaxy proposed by Ashe & Watrous (1984) was successfully applied to the larva of *P. punctipennis*. It may most probably be also useful for larvae of other Omaliinae species, however this will require further research covering more taxa.

**TABLE 2.** Some differences and similarities in chaetotaxy between *Phloeonomus punctipennis* and *Dalotia coriaria*.

<table>
<thead>
<tr>
<th>Body part</th>
<th>Distinctive setae for:</th>
<th>Number of homologous setae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. punctipennis</em></td>
<td><em>D. coriaria</em></td>
</tr>
<tr>
<td>Head/dorsal</td>
<td>Fm2</td>
<td>P4</td>
</tr>
<tr>
<td>Head/lateral</td>
<td>---</td>
<td>E12, E13, Em3, T2</td>
</tr>
<tr>
<td>Head/ventral</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>Labrum/dorsal</td>
<td>---</td>
<td>L11, Lm2</td>
</tr>
<tr>
<td>Pronotum</td>
<td>---</td>
<td>Da3, Db3, Dc3, L4-5, Dd2, P5</td>
</tr>
<tr>
<td>Mesonotum</td>
<td>L2</td>
<td>Da3, Db3, Dd2, L4, P5</td>
</tr>
<tr>
<td>Anterior femur</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>Anterior tibia</td>
<td>---</td>
<td>----</td>
</tr>
<tr>
<td>Prosternum I</td>
<td>St3, St4</td>
<td>Pr1, Ls1, Ls2, Prehy1, Prehy2</td>
</tr>
<tr>
<td>Abdominal tergite I</td>
<td>A3, L3</td>
<td>A4, Db3, Dd2, P5, L4</td>
</tr>
<tr>
<td>Abdominal tergite VIII</td>
<td>A3, L2, P3, P6,</td>
<td>A2, A4, Dd2, L4, Db3, La1, Pa1</td>
</tr>
<tr>
<td>Abdominal sternite I</td>
<td>&quot;</td>
<td>Ps1, D1, D3, P4</td>
</tr>
<tr>
<td>Abdominal sternite II</td>
<td>&quot;</td>
<td>D1, P4, P5, P6</td>
</tr>
<tr>
<td>Source</td>
<td>The present study</td>
<td>Ashe &amp; Watrous (1984)</td>
</tr>
</tbody>
</table>

From among 14 diagnostic characteristics determined for the mature larva of *Phloeonomus* (see page 3), eight of them (characteristics Nos.: 1, 4, 6, 8, 9, 10, 11, 12) are common with the genus *Phloeostiba*. For the first time, a description of a larva of this genus [based on *P. plana* (Paykull, 1792)] was given by Steel (1970). At the same time, the author pointed to a very high similarity of morphology between the final instars of the two genera:
Phloeonomus and Phloeostiba, in comparison to the larvae of 28 other genera belonging to Omaliinae. The convergence seemed high enough that he virtually resigned from a sound systematization of morphology of the Phloeonomus pusillus larva, despite having the study material available. He concluded that the illustrated structures of the larva of Phloeostiba plana: head, antenna, labrum, mandibles, maxilla and labium, are virtually identical to those in P. pusillus. During the analysis, however, he did not take into account most of the morphological details, including ultrastructures taken into account by the authors of the current description of a P. punctipennis larva. In the light of our research, several differences exist between the genera listed above and are as follows: (a) sensory appendage of antennal article II: slightly curved inside, equal in length of article III in Phloeonomus (Phs) or straight, slightly shorter than article III in Phloeostiba (Pha); (b) anterior margin of labrum, surface between setae Lm1 and Ld1: slightly convex in Phs or concave in Pha respectively; (c) dorsal side of mandibles: with row of saw-like teeth along the inside edge in Phs or without such row of teeth in Pha; (d) urogomphi: almost equal in length to abdominal segment X in Phs or slightly longer than abdominal segment X in Pha; (e) at least some long setae on abdomen: bifurcate near top in Phs or all setae simple in Pha. Whereby in the case of the "c" difference, it is unclear whether the lack of such characteristic in the Pha larva is an actual characteristic or whether the author only did not show it on the illustration (which is not very detailed).

Despite those several differences, large similarity in the external morphology of larvae of these genera is a fact that clearly points to their close relationship. All the more because until very recently, the genus Phloeostiba did not have an established systematic position. In the nineties, it was considered by some authors to be a subgenus of the genus Phloeonomus (Herman 2001). Currently Phloeostiba is listed as a separate genus. However, the results of our studies on larval development stages suggest that this position should be reviewed and, perhaps, its rank reduced to the level of subgenus. However, the final conclusions in this respect should be supported with more comprehensive research, including molecular analyses.

On the example of P. punctipennis, the differences in external morphology were for the first time determined between the early (L1) and late (L3) larval development instars for the Omaliinae sub-family. They refer only to the proportion of length of individual parts of antennae and legs as well as length proportion of urogomphi and their subapical setae. No differences were demonstrated between respective larval development stadia in terms of the chaetotaxy analyzed in detail in this study, which came as a major surprise. All the more because such differences, to a lesser or greater degree, occur in the case of other subfamilies of Staphylinidae, such as Paederinae, Staphylininae, Oxytelinae or Aleocharinae (Ashe & Watrous 1984; Staniec 1997; Staniec & Pietrykowska-Tudruj 2008; Staniec et al. 2016). The study also revealed the lack of egg bursters, structures that help young larvae to hatch. These elements are present on the abdominal tergites, sometimes also on the first larva abdominal tergites in all known representatives of Oxytelinae and Aleocharinae (Ashe 1986; Staniec 1997). Whether the lack of variability of chaetotaxy between instars and egg bursters in early instars is a characteristic also of other Omaliinae or the entire subfamily, one should determine in further research of larvae covering a larger number of species of those staphylinids.

Considering the feeding preference, one could presume that the food of P. punctipennis is, in most part, composed of various microscopic fungal species that develop under moist bark or in decomposing old polypores. This presumption is in line with our observations of individual fungal spores (probably) visible in some scan images of part of the studied larva’s mouthparts (Fig. 18a; Sp). This notion is also in line with most of the existing general information concerning the trophic aspects of this staphylinid species (Alexander & Anderson 2012; Sawoniewicz 2013, 2015). Similar feeding preferences are cited by Crowson (1982) for a related Phyllodreopoidea crenata (Omaliinae) inhabiting the same microhabitats as the studied species. In turn, Lipkow & Betz (2005) list Omaliinae in the group of several subfamilies of Staphylinidae the representatives of which are at least facultative mycophages. According to classification of Lawrence (1989), who proposes two types of mycophagy for members of Staphylinidae, the larvae of P. punctipennis (probably also adults) can be classified as microphage staphylinids. This ecological type involves species which usually feed on very small particles or loosely organized food mass consisting of spores, hyphae or highly decomposed animal or plant tissue. It seems that presumably a relatively broad feeding spectrum of the studied larva is reflected in the general morphology of its mouthparts.

In general the structure of the mouthparts of the P. punctipennis larva includes all characteristics of the ground plan of staphylinid mouthparts of larvae cited by Betz et al. (2003). The exception here is the presence of a relatively well-developed membranous mandibular prostheca (Pst) (Figs 12, 19, 19a, 20). According to Betz et al. (2003) it is a part that helps in the transport of food towards the mouth opening and prevents its escape from the
processing area (pa) (Fig. 12; pa marked by arrows). Apart from the genus Phloeonomus, among the known Omaliiniae larvae, this well-developed structure has been described only for the genera Lathrimaeum and Phloeostiba (Pototskaya 1967; Steel 1970). A very specific element of the mouthparts of a larva is the row of saw-like teeth, located along the inside edge in both mandibles (Figs 20, 20b). This structure, in combination with other parts, e.g., epipharynx, may constitute a surface used for rubbing hard food particles, e.g., spores. In turn, the sharp internal edges of mandibles most probably help in breaking down (cutting into shorter sections) of soft organic matter components, e.g., fungal hyphae, while the subapical teeth facilitate scooping various foods from the ground. A further characteristic element of the mouthparts of P. punctipennis is a spatulate and bifurcate tooth (Bt) located on each mala (Figs 21, 21b, 21c). Due to its morphology and posterior location with respect to other mouthparts (Figs 12, 18a), this structure may be helpful at the final stage of transporting (pushing) the food that had already been broken down towards the pharynx. An identical role, but at an earlier stage of food uptake, is performed probably by various posteriorly directed cuticular processes (“cram-brushes”) that are present in relatively high numbers on the epipharynx and hypopharynx (Figs 12, 18, 18a, 23, 24). The detailed morphology of the numerous structures of the mouthparts used for transporting food towards the mouth in the larvae and adults of other representatives of Staphylinidae have been described earlier by Betz et al. (2003) and Lipkow & Betz (2005).

Within the group of microphage staphylinids, the feeding accommodations expressed in the morphology of mouthparts may be even more advanced than in the case of P. punctipennis. The best known species in this scope are those that feed primarily or exclusively on spores (Ashe 1993; Leschen 1993; Betz et al. 2003; Lipkow & Betz 2005). The adaptations of spore-feeder species pertain mainly to the development of specific mouthpart details for gathering (e.g., details resemble brushes, brooms, combs, rakes) and grinding (e.g., rasps surface) of spores (Betz et al. 2003).

Various instars (L1-3) and adult individuals of P. punctipennis were captured on 17th November, which points to the possibility of developmental stages accommodated for wintering. This thesis is in line with earlier assumptions of Steel (1970) who collected larvae in England together with adults of this species in mid-March and at the beginning of April from under beech bark.

Thus far, the data available in the literature clearly point to the close relationship between P. punctipennis and deciduous trees (Steel 1970, Burakowski et al. 1979; Koch 1989). However, in the light of new data, these relationships are not as unequivocal anymore. In mountainous and sub-mountainous areas in the south of Poland, the species may be found in relative abundance also under the bark of fir trees, typically together with various other saproxylic beetles (Melke; verbal information). Apart from habitats located under the bark, this staphylinid also inhabits, together with other beetles, in sporocarps of polypores (Burakowski et al. 1979); however in these microhabitats, within the group of associated insects, no instars of the mycetobiontic C. castaneus were found. With current knowledge, it is difficult to determine the actual ecological links between P. punctipennis and C. castaneus. Perhaps, the tunnels bored in the sporocarps of polypores through various instars of this minute tree-fungus beetle create adequate conditions for the development of microscopic fungi which constitute food for P. punctipennis larvae. This hypothesis, however, should be confirmed with more detailed studies.

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References


