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Ekologie herbivorních a saproxylických brouků (Coleoptera)

Filip Trnka

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Školitel: prof. MVDr. Emil Tkadlec, CSc.
Konzultant: RNDr. Tomáš Kuras, Ph.D.

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ABSTRAKT

Trend poklesu biodiverzity je v současné době nezvratně dokázaným faktem. Abychom byli schopní tento pokles zastavit nebo alespoň zpomalit, je nezbytně nutné znát podrobně jak biologii a ekologii druhů, tak na základě relevantních informací potom umět tvořit modely, které nám mohou pomoci předvídat, které druhy mohou být v budoucnu náchylnější k vymření.

První část této práce si klade za cíl predikovat ohrožení herbivorních brouků na skupině nosatcovitých (Curculionoidea) na základě jejich life history a také ekologických vlastností jejich živných rostlin. Analýza odhalila, že mezi nejvíce ohrožené patří monofágní, nelétavé, teplomilné, světlomilné, suchomilné a hygrofilní druhy s vazbou na otevřené biotopy. Obdobně je to i s vlastnostmi živných rostlin, které vysvětlují ohrožení nosatcovitých. Tyto rostliny lze charakterizovat jako xerotermofilní a hygrofilní vyskytující se na biotopech s minimem dusíku. Z analýzy dále vyplývá, že nosatci, jejichž živné rostliny jsou obecně na ústupu a mají nízkou abundanci na stanovišti, jsou více náchylní k vyhynutí. A v neposlední řadě jsou ohroženější nosatci s vazbou na vodní rostliny a keříčkovitou vegetaci. Life history charakteristiky nosatců se ze všech studovaných vlastností ukázaly pro predikci ohrožení důležitější než ekologické vlastnosti rostlin. Nicméně vlastnosti živných rostlin se zdají být důležité a měly by být použity pro predikční modely herbivorních skupin živočichů (Příloha I).

Druhá část práce se zaměřila na základní výzkum a odhaluje bionomii několika druhů nosatců, jejich živné rostliny, larvální stádia, stanovištní preference, ekologické a morfologické adaptace. Tyto výsledky významně přispívají k základnímu výzkumu, ale budou mít i praktické důsledky pro ochranářskou biologii a mohou být využity k biologické kontrole invazních druhů rostlin (Příloha II–V).

Klíčová slova: Coleoptera, Curculionoidea, larvální stádia, life history, morfologie, nosatci, ochrana přírody, riziko vyhynutí, živné rostliny

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ABSTRACT

Nowadays biodiversity decline is the indisputable fact. To stop or at least slow down this trend a detailed knowledge of species biology and ecology is essential. Consequently, the relevant biological information may help us create models for prediction of species extinction risk in the future.

First part of this doctoral thesis is focused on the prediction of the extinction risk of the herbivorous beetles from the weevil group (Curculionoidea) based on their life history traits and the ecological traits of their host plants. Our analysis revealed the most endangered species are the monophagous and flightless ones that belong to the thermophilic, heliophile, xerophilous or hygrophilous species of open habitats. For the ecological host plant traits explaining the extinction risk of the weevils the situation is almost the same. Those plants could be ranked among xerothermophilous and hygrophilous species occuring on the low nitrogen biotopes. According to our analysis weevils whose host plants are declining and have low abundances at their biotopes are more vulnerable to the extinction risk. Also the weevils connected to aquatic plants and shrubby vegetation are more vulnerable. Weevil life history traits were proved to be more imporant factor for the prediction of the extinction risk than the ecological host plant traits. Nevertheless, the ecological host plant traits are also important and should be used for the prediction models of the herbivore species groups (Appendix I).

Second part of this doctoral thesis is focused on the basic research of the bionomy of a few weevil species, their host plants, larval stages, habitat preferences and morphological and ecological adaptations. The results significantly contribute to the basic knowledge about these species but they may also have a practical use in conservation biology and biological control of invasive plants (Appendices II-V).

Key words: Coleoptera, Curculionoidea, extinction risk, host plants, immature stages, life history, morphology, nature conservation, weevils

OBSAH

SEZNAM ORIGINÁLNÍCH PUBLIKACÍ

Předložená dizertační práce je založená na níže uvedených publikacích, které jsou citovány nebo označeny jako Přílohy I–V. Autor se podílel na designu studie (I, II, III, IV, V), na sběru dat (I, II, III, IV, V), analýze dat (II, III, IV, V), interpretaci výsledků (I, II, III, IV, V), psaní manuskriptu (I, II, III, IV, V), revizích a korekturách textu (I, II, III, IV, V) a na recenzním řízení a komunikaci s redakcí časopisu (I, III, IV). Prohlášení spoluautorů o autorském podílu uchazeče na publikacích je v Příloze VI.

Příloha I:

Trnka F., Stejskal R., Šipoš J. & Kuras T. (2019): Weevils: suitable candidates for prediction of herbivorous insect extinction risk? Submitted to *Biological Conservation*.

Příloha II:

Stejskal R., Trnka F. & Skuhrovec J. (2014): Biology and morphology of immature stages of Coniocleonus nigrosuturatus (Coleoptera: Curculionidae: Lixinae). *Acta Entomologica Musei Nationalis Pragae* 54: 337–354.

Příloha III:

Trnka F., Stejskal R. & Skuhrovec J. (2015): Biology and morphology of immature stages of Adosomus roridus (Coleoptera: Curculionidae: Lixinae). *Zootaxa* 4021: 433–446.

Příloha IV:

Trnka F., Stejkal R. & Skuhrovec J. (2016): The morphology of the immature stages of two rare Lixus species (Coleoptera, Curculionidae, Lixinae) and notes on their biology. *ZooKeys* 604: 87–116.

Příloha V:

Skuhrovec J., Stejskal R., Trnka F. & di Giulio A. (2017): Velcro-like system used for fix a protective faecal shield on weevil larvae. *PLoS ONE* 12 (1): e0170800.

PODĚKOVÁNÍ

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ÚVOD

Celosvětový pokles biodiverzity je už po desítky let aktuálním tématem (Thomas & Abery 1995, Pimm & Raven 2000). Teprve v posledních letech je ale diskutován i významný pokles biodiverzity bezobratlých a obzvláště hmyzu (Sánchez-Bayo & Wyckhuys 2019). Ochrana biologické rozmanitosti na Zemi je mezinárodním zájmem (*Convention on Biological Diversity*; CBD 2014). Tuto úmluvu podepsali zástupci všech států světa, pro které je následně ochrana všech živých organismů a jejich prostředí zavazující. Masivní úbytek druhové diverzity, který ve 21. století zažíváme (Butchart et al. 2010, Sánchez-Bayo & Wyckhuys 2019), se neprojevuje pouze poklesem či ztrátou populací vzácných a ohrožených druhů, ale také rychlým ochuzováním diverzity regionální a lokální (Sax & Gaines 2003, Edgar & Samson 2004) a snižováním abundancí běžných druhů (Hallmann et al. 2017).

Bezobratlí obecně jsou jako skupina z hlediska ohroženosti prozkoumáni mnohem méně (McKinney 1999, Donaldson 2016) a jsou mnohem více ohrožení než obratlovci (Thomas et al. 2004). Přítomnost hmyzu v ekosystémech je přitom klíčová nejen pro člověka, protože poskytují jedny z nejzákladnějších ekosystémových služeb (Jankielsohn 2018) jako je např. opylování, management škůdců nebo rozklad organické hmoty (Losey & Vaughan 2006). Právě skupiny herbivorních a saproxylických druhů hmyzu patří k těm, které se těchto procesů účastní a hrají v ekosystémech i další důležité role jako predátoři či paraziti (Fonseca 2009). Pro zachování těchto ekosystémových služeb je ochrana biodiverzity klíčová.

Jen ve Spojených Státech bylo vyčísleno, že pokud by došlo k odstranění čtyř základních ekosystémových služeb od volně žijícího hmyzu (rozklad organické hmoty, kontrola škůdců, obohacování půdy organickou hmotou, opylování), došlo by k roční škodě okolo 57 bilionů dolarů (Losey & Vaughan 2006). Dle autorů to významně ospravedlňuje náklady na výzkum a ochranu těchto druhů a jejich prostředí.

Vztahy mezi organismy a jejich životním prostředím se zabývá věda nazývaná obecně ekologie. Podle zaměření se věnuje jedincům, populacím, celým společenstvům a ekosystémům. Právě pochopení co nejvíce vztahů v ekosystémech a důkladná znalost biologie a ekologie jednotlivých druhů, populací a společenstev je klíčová pro ochranu a management druhů a biotopů, a tudíž pro úspěšnou ochranu přírody obecně (Pickett et al. 1994, Cardoso et al. 2011).

OHROŽENÍ A OCHRANA HMYZU

Hlavními příčinami ohrožení hmyzu je izolace, fragmentace a destrukce biotopů (Fischer & Lindenmayer 2007), také jejich znečištění (Dudley & Alexander 2017) a v menší míře také globální změna klimatu (Thomas et al. 2004, Urban 2015). Tyto příčiny jsou bezprostředně způsobovány růstem lidské populace a její činností, především intenzivním hospodařením, které se v posledních desetiletích násobně stupňuje (Krebs et al. 1999). Vyjma samotné intenzifikace zemědělství a lesnictví je velkým problémem upouštění od tradičních forem hospodaření, a tím pádem ztráta heterogenní krajiny (Benton et al. 2003). Zejména v případě nelétavých druhů je homogenní krajina s izolovanými a fragmentovanými biotopy neprostupná a snižuje se šance na rekolonizování vhodných biotopů (Thomas et al. 2001). Upouštěním od extenzivní seče a pastevectví se stávají velmi ohrožené různé typy přirozených a polopřirozených otevřených biotopů (Fischer et al. 1996, Dover et al. 2003). Také pastva v lesích nebo výmladkové hospodaření vytváří vhodné podmínky pro dnes ohrožené druhy (Saarinen et al. 2005, Vodka et al. 2009, Horák et al. 2012). Fakticky tedy často vymírají i specializované druhy, které jsou naopak závislé na extenzivní nebo specifické hospodářské činnosti člověka, kterou vznikají typické mikro a makrohabitaty (Roslin et al. 2009, Thomas et al. 2009).

Dle autorů Sánchez-Bayo & Wyckhuys (2019) dochází v posledních letech k tak dramatickému poklesu biodiverzity hmyzu, že by to podle odhadů mohlo v dalších několika příštích desítkách let způsobit vyhynutí až 40 % všech druhů hmyzu. V suchozemských ekosystémech považují za nejohroženější skupiny motýly, blanokřídlé a brouky. Řešením je podle nich hlavně přehodnocení současných zemědělských praktik - hlavně snížení spotřeby syntetických pesticidů a jejich nahrazení více udržitelnými, ekologickými praktikami. V posledních letech publikoval Hallmann et al. (2017) výsledky 27leté studie, která odhalila až 76% pokles biomasy létajícího hmyzu v několika německých chráněných územích. To znamená průměrnou ztrátu 2,8 % hmyzu za rok, a to ještě v územích, která by měla být zachovalejší a před lidskými aktivitami chráněnější. Tyto klesající trendy výskytu jak biomasy, tak počtu druhů hmyzu v poslední době naznačují, že jsme možná svědky dalšího možného velkého vymírání (Thomas et al. 2004).

Brouci jako nejpočetnější řád ze skupiny hmyzu zajišťují v ekosystémech mnoho základních ekosystémových služeb, jako např. kontrola škůdců nebo koloběh organické hmoty. Např. pro čeleď střevlíkovitých se uvádí jako nejvážnější příčiny úbytku zánik biotopů, zvýšená eutrofizace prostředí díky intenzifikaci v zemědělství a urbanizace krajiny (Koivula 2011, Nolte et al. 2019). Saproxylický hmyz je v ekosystémech důležitý pro svou schopnost rozkládat dřevní hmotu, a tak vracet živiny opět do oběhu (Grove 2002). Některé z těchto taxonů jsou zároveň významnými opylovači (Stefanescu et al. 2018).

Pokles počtů hmyzu se zdá být podobný v tropických i temperátních oblastech (Sánchez-Bayo & Wyckhuys 2019). Co se týče temperátních oblastí, nejvíc zdokumentovaných případů poklesu počtu hmyzu se týká Velké Británie (60 % druhů), následuje Severní Amerika (51 % druhů) a Evropa (44 % druhů). Neméně znepokojujícím faktem také je to, že neklesají jen počty vzácných druhů hmyzu s úzkou ekologickou nikou, ale klesají i početnosti druhů, které ještě nedávno byly rozšířené velmi široce. To ukazuje na skutečnost, že poklesy počtu hmyzu nesouvisí jen s ničením konkrétních biotopů, ale celá skupina je ovlivněna společnými poklesovými trendy (Gaston & Fuller 2007).

Antropogenní vlivy způsobují změny hmyzích společenstev k těm na druhy chudším a s převládajícími generalisty (White & Kerr 2007). Zatímco nespočetně druhů vymírá, několik dalších postupně obsazuje volné niky a začíná svůj výskyt rozšiřovat. To může sice pomoct zachovat některé ekosystémové služby, ale není jasné, jak do budoucna budou společenstva reagovat. Postupně se totiž stávají více uniformními a méně rozmanitými (Memmott et al. 2004).

K řešení této situace s neustále narůstajícím úbytkem hmyzu navrhuje Samways (2007) šest základních principů: i) udržovat rezervace, především jako zdrojové biotopy pro specialisty, ii) udržovat krajinnou rozmanitost co nejvíce, iii) zmenšit rozdíly mezi zbytkovými přírodními místy a těmi zničenými, iv) mimo rezervace šetřit půdu, dále ji neničit, v) simulovat přírodní přirozené podmínky a disturbance a vi) propojovat mezi sebou zbylé kvalitní plochy biotopů. Těchto šest principů poskytuje hrubý nástin ochrany hmyzích společenstev. Pak se musí zároveň přidat i jemnější principy konkrétní druhové ochrany.

Snahy identifikovat a následně vyhodnotit oblasti s vysokým rizikem vymírání hmyzu jsou často podkopány právě zásadním nedostatkem informací o druzích hmyzu (Bossart et al. 2002). Hmyzí druhy mohou oproti jiným skupinám fungovat jako výborné ekologické indikátory či se účastnit procesů environmentální degradace. Je to skupina velmi početná a oproti rostlinám nebo ostatním živočišným skupinám se na skladbě biodiverzity podílí velmi značně. Hmyz je široce rozšířený, reprezentuje pestrou škálu životních strategií, vyskytuje se téměř ve všech ekologických nikách, naplňuje široké spektrum funkčních rolí, je klíčový pro udržitelnost ekosystémů.

PREDIKCE OHROŽENÍ

Pochopením hlavních příčin úbytku biodiverzity je nezbytné předem predikovat, jaké druhy budou v budoucnu ohroženy. Na pochopení těchto mechanizmů stojícími za snižováním diverzity je zapojeno mnoho faktorů (Fischer et al. 2003), jak vnějších (např. ztráta biotopů, změna klimatu), tak vnitřních (life history). Druhy, které mají podobné life history, mají také pravděpodobně podobnou citlivost vůči změnám v prostředí a také mají podobnou šanci na riziko vymření (Moretti et al. 2017).

Většina studií se soustředí na predikci ohrožení analýzami červených seznamů a vlastností druhů a jsou zaměřeny především na obratlovce (cf. Purvis et al. 2000, Krüger & Radford 2008) nebo poměrně dobře prostudované či charismatické skupiny hmyzu jako jsou denní motýli, vážky, motýlice, saproxyličtí brouci nebo střevlíci (Kotiaho et al. 2005, Clausnitzer et al. 2009, Seibold et al. 2015, Nolte et al. 2019). Tyto výsledky jsou proto jen částečně přenosné na další skupiny hmyzu a mohou být zkreslené, proto je tedy nutné studovat i další skupiny bezobratlých.

Mezi typické vlastnosti predikce ohrožených druhů denních a nočních motýlů patří úzká potravní nika, malá mobilita, omezená distribuce zdrojů, krátká doba letu, nízká reprodukce a vazba na otevřená stanoviště nebo světlé lesy (Kotiaho et al. 2005, Matilla et al. 2006, Öckinger et al. 2010, Pavlikova & Konvicka 2012). Kotiaho et al. (2005) překvapivě zjistili, že populace ohrožených druhů mají větší hustotu populací než druhy považované za běžné a neohrožené, a také uvádí, že většina ohrožených druhů denních motýlů ve Finsku má sice málo populací, ale ty jsou zároveň relativně početné a životaschopné. Model autorů Seibold et al. (2015) predikuje vlastnosti ohrožení u saproxylických druhů, největší extinkční riziko mají druhy nížin s větším tělem, s vazbou na větší průměry mrtvých nebo odumírajících stromů ve světlých listnatých lesích. U predikce ohrožení střevlíkovitých hraje největší roli úzká vazba na stanoviště, malý areál rozšíření, větší velikost těla a vazba na otevřené biotopy (Nolte et al. 2019). Lze tedy říci, že většina ohrožených herbivorních nebo saproxylických druhů či druhy, kterým je ohrožení v budoucnu predikováno, má úzkou vazbu na stanoviště, kterým jsou především otevřené biotopy nebo světlé lesy.

Až dosud se studie zaměřovaly na porovnání life history ohrožených i neohrožených druhů hmyzu směrem k propojení jejich klasifikace v červeném seznamu s jejich life history. Ale mezi faktory, které ovlivňují riziko vyhynutí a ohroženost, by měly být zapojeny i další parametry prostředí, jako např. živné rostliny u herbivorních druhů. Herbivorní druhy fungují v rámci společenstev jako významný prvek, který ovlivňuje celý potravní systém (Polis & Winemiller 1996), což bylo zatím ve studiích zabývajících se predikcí ohroženosti převážně ignorováno.

V rámci naší studie k predikci ohrožení herbivorních skupin hmyzu (Příloha I.) jsme jako vhodnou skupinu vybrali nosatcovité brouky (Curculionoidea), kteří jsou považovaní za indikátory zachovalosti prostředí (Benedikt et al. 2010) a jedná se zdaleka o největší skupinu herbivorů (Oberprieler et al. 2014). Ve studii jsme testovali nejen life history téměř 1 000 druhů nosatcovitých ale také ekologické vlastnosti jejich živných rostlin (Ellenberg et al. 1992) společně s analýzou červeného seznamu nosatců (Benedikt & Strejček 2005). Fylogeneticky upravená analýza (Felsenstein 1985, Harvey & Pagel 1991) nám umožnila identifikovat life history charakteristiky, které vedou k poklesu populací a predikují, které druhy mohou být potenciálně ohroženy.

Naše analýza odhalila, že mezi herbivorními druhy nosatců patří k nejvíce ohroženým skupinám ty druhy, které jsou monofágní, nelétavé, teplomilné, světlomilné, suchomilné a vlhkomilné a žijící na otevřených stanovištích. Z živných rostlin nejlépe vystihují ohroženost druhů nosatců ty, které jsou suchomilné a teplomilné nebo mokřadní s vazbou na otevřené biotopy. Z analýzy dále vyplývá, že nosatci, jejichž živné rostliny jsou obecně na ústupu, jsou vzácné a mají nízkou abundanci na stanovišti, jsou více náchylní k vyhynutí. A nakonec hydrofyty (vodní rostliny) a chamaefyty (polokeře) předurčují více ohrožené nosatce. V celkové analýze všech používaných charakteristik se ukázalo, že life history charakteristiky druhů nosatců jsou pro predikci ohrožení více důležité než ekologické vlastnosti jejich živných rostlin. Zároveň jsou ale ekologické charakteristiky živných rostlin důležité a měly by v modelech predikce ohrožení herbivorních druhů být jednoznačně používány dohromady s charakteristikami herbivorů.

Studie predikce rizika vyhynutí jsou někdy kritizovány pro nedostatek propojení svých výsledků s praktickou ochranou druhů (Cardillo & Meijaard 2012). Ke zpřesnění výsledků studií predikce v praktické ochraně druhů je navrhováno omezení geografického a taxonomického zaměření a vypracování jasných závěrů (Cardillo & Meijaard 2012). K vypracování jasných závěrů je třeba znát přesné měřítko studie a konkrétní detaily predikovaných druhů. Tudíž pro druhy ohrožené antropogenními změnami habitatu jsou nejdůležitější dobře vydefinované charakteristiky, které popisují zdroje, na kterých jsou tyto druhy závislé. To může pomoci identifikovat omezení těchto zdrojů, které zvyšují jejich riziko vyhynutí (Gossner et al. 2013).

Jeden z nejzákladnějších problémů v ohrožení a následné ochraně hmyzu je malá nebo úplná neznalost bionomie druhů (Bossart & Carlton 2002). Pro poměrně mnoho druhů nejsou naneštěstí k dispozici ani základní ekologická data. Nedostatek těchto dat může plynout z toho, že dnešní biologické vědy preferují studium teorií spíš než základní ekologické studie (Mattila et al. 2006). Proto je i pro predikci ohrožení hmyzu naprosto zásadním krokem co nejpřesnější znalost bionomie jednotlivých druhů (Příloha II–V), z jejichž ekologických charakteristik predikce následně vycházejí.

NOSATCOVITÍ BROUCI

Nadčeleď nosatcovitých brouků (Curculionoidea) je celosvětově nejpočetnější a nejdiverzifikovanější skupinou brouků, pravděpodobně i veškerého hmyzu (Oberprieler et al. 2014). Počet popsaných taxonů je odhadován na 100 000 (Astrin et al. 2012), celkové odhady jsou okolo 220 000 druhů (Oberprieler et al. 2007). Diverzita nadčeledi bude pravděpodobně i násobně větší, jelikož v poslední době bylo popsáno několik velmi diverzifikovaných kryptických rodů s desítkami druhů (cf. Anderson 2010, Riedel 2010). V moderních fylogenetických klasifikacích se nadčeleď skládá ze sedmi čeledí – Nemonychidae, Anthribidae, Belidae, Attelabidae, Caridae, Brentidae a největší čeledi, do které náleží většina druhů, Curculionidae (Oberprieler et al. 2007, McKenna et al. 2009). Dle některých autorů je rozlišováno i více čeledí (Thompson 1992, Bouchard et al. 2011). Svojí početností jsou nosatcovití považováni za evolučně nejvyspělejší skupinu brouků (Crowson 1967). Skupina je celosvětově rozšířena a obývá většinu typů suchozemských a sladkovodních biotopů od arktické zóny po tropické lesy, kde diverzita dosahuje maxima (Oberprieler et al. 2007).

Nosatcovití jsou téměř výlučně herbivorní brouci často s monofágní nebo oligofágní vazbou především na krytosemenné rostliny (Magnoliophyta), méně často na nahosemenné (Pinophyta). Část druhů je saproxylických či fungivorních (Anthribidae, Cossoninae, Cryptorhynchinae, Scolytinae) s vazbou na odumírající dřevní hmotu nebo listový opad (Oberprieler et al. 2014). Odlišný vývoj mají větevníčci rodu *Anthribus*, respektive jejich larvy (Hoebeke & Wheeler 1991), kteří jsou predátoři puklicovitých (Coccidae) a mšicosavých (Sternorrhyncha). Larvy australských nosatců rodu *Tentegia* požírají klokaní trus (Wassel 1966) a brazilský nosatec *Ludovix fasciatus* je predátorem sarančích vajíček (Zwölfer & Bennett 1969). Některé druhy ze skupin Brentidae a Curculionidae jsou myrmekofilní (Marshall 1946, Sforzi & Bartolozzi 2004). U části druhů z čeledi Attelabidae je typický parazitismus, kdy dospělci obsazují již existující smotky jiných druhů zobonosek a kladou do nich vajíčka (Dieckmann 1974, Hamilton 1998). Někteří nosatcovití jsou také významnými opylovači cykasů, palem a primitivních krytosemenných rostlin a sehravají hlavní roli v přežití těchto ohrožených druhů (Oberprieler 2004).

Vývoj nosatcovitých probíhá v kořenech, stoncích, listech, trouchnivějícím dřevě, květech nebo v plodech. Vetší část druhů je oligofágních s vazbou na rod nebo čeleď rostlin, menší část tvoří monofágní a polyfágní druhy. Jednu živnou rostlinu dokáže obsadit několik druhů, např. na běžné pumpavě obecné (*Erodium cicutarium*) probíhá larvální vývoj čtyř druhů nosatcovitých – na květních pupenech se vyvíjí *Limobius borealis*, na listech *Brachypera dauci*, v kořeni *Lixus vilis* a v půdě v blízkosti řapíků listů *Coniocleonus nigrosuturatus* (Koch 1992, Příloha II). Vývoj jednotlivých druhů na jedné živné rostlině není oddělen pouze prostorově, ale v některých případech i časově do různých období roku (cf. Freude et al. 1981, 1983).

Mnoho druhů je ve střední Evropě považováno za významné bioindikátory zachovalosti biotopů (Benedikt et al. 2010) a velká část z těchto taxonů je celoevropsky ohrožených (např. Geiser 1998, Sprick et al. 2003, Stejskal et al. 2017). Podobně jako u jiných herbivorních bezobratlých je vzácnost či ohrožení daná úzkou vazbou na zachovalé biotopy, respektive specifické sekundární biotopy (Stejskal & Trnka 2013, Příloha II, III, IV) nebo vzácností či lokálním výskytem živných rostlin (Mattila et al. 2008). Převažující nejohroženější skupinou nosatcovitých jsou druhy teplomilné, suchomilné a také hygrofilní s vazbou na otevřená stanoviště (Příloha I).

Některé skupiny nosatcovitých, převážně monofágů až úzkých oligofágů, jsou považovány jako vhodné pro biologickou kontrolu nepůvodních a často velmi invazních druhů rostlin (Muniappan et al. 2009). Mezi nejúspěšnější skupiny v potlačování těchto rostlin patří tribus Tanysphyrini (Oberprieler 2014). Asi nejznámějším případem je použití dvou druhů nosatců *Neochetina eichhorniae* a *N. bruchi* pro potlačení jihoamerické tokozelky nadmuté (*Eichhornia crassipes*), tzv. vodního hyacintu, který patří k nejinvazivnějším rostlinám v tropických oblastech. Tito nosatci jsou schopni velmi dobře redukovat květy, semena a celkově efektivně oslabovat populace této rostliny (De Groote et al. 2003). Dalším velmi zajímavým příkladem je i potlačování severoamerické vodní kapradiny azoly americké (*Azolla filiculoides*) nosatcem *Stenopelmus rufinasus* v jižní Africe, který je považován za jeden z nejúspěšnějších příkladů v historii biologické kontroly, kdy nosatec kapradinu totálně potlačil během 10 let od jeho vysazení na invadovaná stanoviště (Hill & McConnachie 2009).

Je třeba zmínit, že ne všechny biologické kontroly s využitím nosatců byly úspěšné. Běžný evropský nosatec *Rhinocyllus conicus* byl do severní Ameriky introdukován za účelem potlačení invazních bodláků (*Carduus* spp.) a pcháčů (*Cirsium* spp.). Naneštěstí *R. conicus* začal potlačovat nepůvodní druhy, ale také původní pcháče jako je *Cirsium canescens, C. hillii, C. pitcheri* nebo *C. undulatum* (Sauer & Bradley 2008). U pcháče *C. canescens* je nosatec schopný zredukovat produkci semen až o 85 % a byla vyslovena hypotéza, že by tento dosud běžný druh pcháče mohl v brzké době vyhynout (Rand & Louda 2006). Na těchto pcháčích žije také několik původních druhů hmyzu z různých řádů a některé tento nosatec začal postupně vytlačovat a negativně ovlivnil jejich populace (Louda et al. 2003).

EKOLOGIE LARVÁLNÍCH STÁDIÍ NOSATCOVITÝCH

Nejběžnější typ larvy nosatcovitých brouků je slabě pigmentovaný a sklerotizovaný, bez končetin a tvaru písmene C (Scherf 1964). Larvy nosatcovitých žijí jak endofytním, tak endofágním způsobem života, ale koncepty endo/ektofytní a endo/ektofágní nejsou identické a je třeba je správně rozlišovat. Endofytní druhy žijí uvnitř rostlinných pletiv, kdežto označení endofágní odkazuje na žír uvnitř jakéhokoli substrátu (Oberprieler et al. 2014). Pouze několik taxonů má oba typy larev - jak endofytní, tak ektofytní, např. žír larev prvních instarů probíhá uvnitř stonků a později se larva přesouvá ze stonku do půdy, kde si tvoří hliněnou komůrku napojenou na stonek nebo kořen (Oberprieler et al. 2014).

Endofytní (a také endofágní) je většina larev nosatcovitých brouků, ale některé skupiny jsou i ektofytní. Ektofytní a také endofágní larvy typicky žijící v půdě mají např. Entiminae (12 000 druhů v 1 370 rodech), pro ně je typická strategie života larev mimo živnou rostlinu v blízkosti jejích kořenů, kterými se živí (Marvaldi et al. 2014). Ektofágní (a také ektofytní) jsou larvy žijící volně na exponovaných, vnějších částech rostlin, a jsou tak adaptovány k pohybu na rostlině. Pro pohyb slouží rudimentové hrudní výběžky nebo panožkovité výběžky, které nese posledních šest zadečkových článků (Oberprieler et al. 2014, Příloha V). Mezi tyto skupiny patří např. Nemonychidae, Ectemnorhinini, většina Cionini, Gonipterini, Hyperini a také někteří Cyclominae a Ceutorhynchini (Oberprieler et al. 2014). Některé druhy mají i larvy endo/ektofytní a tyto strategie dokáží během larválního vývoje střídat (cf. Hill & McConnachie 2009). U taxonů s endofytními larvami samičky obvykle nakousávají rostlinná pletiva a tvoří jamku, do které kladou vajíčka, naopak druhy s ektofytními larvami kladou vajíčka do půdy nebo do poškozených částí rostlin (Oberprieler 2010). Kuklení probíhá přímo uvnitř živných rostlin, v hliněné komůrce v půdě a např. u Hyperini ve specifickém kokonu připevněném nejčastěji z vnějšku živné rostliny (cf. Oberprieler et al. 2014).

Larvy Nemonychidae jsou bionomicky převážně vázany na jehličnany a živí se jejich pylem a sporofyly, menšina druhů s vazbou na byliny se živí plody, ve kterých vyžírají semena a kuklí se v kulovitých kokonech (Kuschel 1993). Pro Anthribidae je typický larvální vývoj v kůře nebo dřevě napadeném dřevokaznými houbami. Některé druhy jsou přímo fungivorní, žijící v plodnicích hub (Kuschel 1995). U rodu *Anthribus* jsou larvy predátoři Coccidae a Sternorrhyncha (Hoebeke & Wheeler 1991). Larvy novozélandského druhu *Lichenobius littoralis* se vyvíjejí v lišejnících, které jsou ostřikovány vodopády (Holloway 1982). Urodonidae jsou herbivorní a jejich vývoj probíhá nejčastěji v semenech bylin (Kuschel 1995). Některé skupiny jsou vážnými škůdci skladovaných semen a obilí (Valentine 1999). Pro Belidae jsou typické endofytní larvy žijící pod kůrou větví keřů a stromů, méně potom v květech a plodech (Marvaldi et al. 2006). Attelabidae mají vazbu na dvouděložné rostliny, v jejich zasychajících tkáních se larvy vyvíjejí a často jsou infikovány houbami nebo plísněmi. Samice zobonosek místo k nakladení vajíček předem připravují, buď smotávají listy nebo vykoušou otvor např. do letorostu, pupenu či květu (cf. Oberprieler et al. 2014). Nejmenší čeleď nosatcovitých Caridae má vazbu na jehličnany, vyvíjejí se ve sporofylech a šišticích, larvy se kuklí v půdě (Oberprieler et al. 2007). Největší skupiny Brentidae a Curculionidae mají larvy převážně endofytní žijící ve všech rostlinných částech od kořenů až po pupeny, květy a semena. Některé larvy tvoří hálky nebo minují v listech. Nicméně několik skupin žije ektofytně volně na listech nebo v půdě na kořenech živných rostlin (cf. Oberprieler et al. 2014).

Pokusili jsme se zjistit nebo doplnit biologii a ekologii několika druhů, především v podčeledi Lixinae, které měly málo nebo naprosto neznámou bionomii a ve střední Evropě jsou vzácní nebo v ČR dokonce vyhynulí (cf. Stejskal et al. 2017). V první řadě se podařilo zjistit podrobnou ekologii druhu *Coniocleonus nigrosuturatus*, který žije pravděpodobně monofágně na pumpavě obecné (*Erodium cicutarium*), jeho larvy žijí endofytně mimo živnou rostlinu v hliněné komůrce, odkud probíhá žír stonku a řapíků. Tento způsob života je v rámci Lixinae ojedinělý a zatím ještě nebyl u žádného druhu pozorován (Příloha II). Dále se povedlo potvrdit již známé živné rostliny velmi vzácného druhu *Adosomus roridus* a zjistit, že larvy žijí endofytně v kořenovém krčku a v kořenech. A také podrobně popsat vývoj a ekologii tohoto druhu (Příloha III). V poslední fázi jsme se zaměřili na velmi vzácné druhy *Lixus bituberculatus* a *L. neglectus*, kteří mají společné, že žijí v malých areálech a jsou to ryze evropské druhy. Podařilo se nám zjistit detailní ekologii - u obou druhů jsou larvy endofytní a jsou to pravděpodobně monofágové nebo oligofágové. *Lixus bituberculatus* žije na čekance obecné (*Cichorium intybus*) a vývoj *L. neglectus* probíhá ve šťovíku rozvětveném (*Rumex thyrsiflorus*) (Příloha IV). Posledním studovaným taxonem byl *Eucoeliodes mirabilis* z podčeledi Ceutorhynchinae, u kterého jsme potvrdili už známou ekologii a navíc se podařilo objevit ektofytní larvy, které jsou v rámci nosatcovitých výjimečné, protože na svém těle nesou fekální štít, který je k tělu přichycen mikrotrichiemi. Tato naprosto jedinečná antipredační strategie je u nosatcovitých popsána zcela prvně a byla známa pouze u mandelinkovitých (Vencl & Morton 1999, Příloha V).

Imaturním stádiím nebyla v minulosti věnována velká pozornost a jejich znalost je stále poměrně slabá, protože se výzkum soustředil především na dospělce (Marvaldi 1997). V současné době ale zájem poměrně stoupá a bylo popsáno mnoho larválních stádií např. z podčeledi Ceutorhynchinae (Skuhrovec et al. 2017, Příloha V), Entiminae (cf. Sprick & Gosik 2014, Gosik et al. 2016, 2017, 2019) nebo Lixinae (cf. Nikulina & Gültekin 2011, Gosik & Wanat 2014, Příloha II, III, IV).

Díky vzrůstajícímu poznání larválních stádií mohou být lépe a efektivněji chráněny vzácné druhy, u kterých nebyla známa bionomie. Popisy larev a kukel jednotlivých druhů a jejich srovnání s druhy již známými dokazuje, že je možné identifikovat již nedospělá stadia přesně tak, jak se to dělá v jiných skupinách. V praxi je identifikace druhu podle chaetotaxie poměrně snadná a je rozhodně levnější než použití molekulárních metod (Hirsch et al. 2010). Bohužel je stále ještě k dispozici velmi málo popsaných nedospělých stádií nosatců v porovnání s velkým počtem druhů popsaných dospělců. Hlavním problémem je, že nejsme schopní rozdělit morfologické charakteristiky v této skupině na charakteristiky užívané ve fylogenetice a charakteristiky užívané pro určování druhů. Jakmile bude tato kategorizace dokončená, bude možné použít ji pro fylogenetické studie.

ZÁVĚR

Hlavní příčiny úbytku biodiverzity je nezbytné předem predikovat. Na pochopení těchto mechanizmů stojícími za snižováním diverzity je potřeba zvažovat mnoho faktorů (Fischer et al. 2003). Abychom mohli spolehlivě predikovat ohrožení hmyzu (Příloha I), je nutné co nejpřesněji znát bionomii jednotlivých druhů (Příloha II–V). Bez této znalosti jsou predikce neúplné a ochrana druhů je minimální, pokud neznáme jejich živné rostliny a přesné life history (Bossart & Carlton 2002, Mattila et al. 2006). Dosud se studie zaměřovaly na porovnání life history ohrožených i neohrožených druhů herbivorů směrem k propojení jejich klasifikace v červeném seznamu s jejich life history (cf. Kotiaho et al. 2005, Nolte et al. 2019). Mezi faktory, které ovlivňují riziko vyhynutí a ohroženost, by měly být zapojeny i další parametry prostředí, jako např. živné rostliny u herbivorních druhů. V rámci společenstev jsou herbivoři velmi významní a ovlivňují celý potravní systém (Polis & Winemiller 1996), což bylo zatím ve studiích zabývajících se predikcí ohroženosti převážně ignorováno.

V naší studii k predikci ohrožení herbivorních skupin hmyzu jsme si vybrali jako modelovou skupinu nosatcovité brouky (Curculionoidea), kteří jsou považovaní za indikátory zachovalosti prostředí (Benedikt et al. 2010) a jedná se zdaleka o největší skupinu herbivorů (Oberprieler et al. 2014). Predikci jsme testovali na základě life history nosatců a také ekologických vlastností jejich živných rostlin. Analýza odhalila, že největší riziko vyhynutí mají druhy monofágní, nelétavé, teplomilné, světlomilné, suchomilné a hygrofilní druhy s vazbou na otevřené biotopy. Obdobně je to i s vlastnostmi živných rostlin, které vysvětlují ohrožení nosatcovitých. Živné rostliny lze charakterizovat jako xerotermofilní a hygrofilní vyskytující se na biotopech s minimem dusíku. Nosatci, jejichž živné rostliny jsou obecně na ústupu a mají nízkou abundanci na stanovišti, jsou více náchylní k vyhynutí. A v neposlední řadě jsou ohroženější nosatci s vazbou na vodní rostliny a keříčkovitou vegetaci. Ze všech studovaných charakteristik se jako důležitější pro predikci ohrožení ukázalo life history nosatců než ekologické vlastnosti rostlin. Nicméně vlastnosti živných rostlin se zdají být důležité a měly by být použity pro predikční modely herbivorních skupin živočichů.

Dále jsme se zaměřili na odhalení biologie několika druhů, u kterých byla bionomie buď málo známá (Příloha III, V) nebo úplně neznámá (Příloha II, IV). Našimi sledovanými druhy byli především nosatci z podčeledi Lixinae, kde je stále u několika druhů téměř neznámá bionomie a především živné rostliny. U druhů *Adosomus roridus*, *Coniocleonus nigrosuturatus*, *Lixus bituberculatus* a *Lixus neglectus* se nám biologii a ekologii podařilo objasnit. V dalším případě jsme se zabývali detailní bionomií nosatce z podčeledi Ceutorhynchinae, konkrétně *Eucoeliodes mirabilis*, u kterého se kromě upřesnění biologie podařilo objevit i zajímavou adaptaci proti predátorům. Larva na svém těle nese tzv. fekální štít, který byl do té doby znám pouze u brouků z čeledi mandelinkovitých (Chrysomelidae).

Budoucí detailní výzkumy biologie a morfologie jednotlivých druhů mohou poskytnout zásadní informace pro efektivnější plánování biologické kontroly invazivních druhů rostlin. Téma použití druhů hmyzu jako biologických agentů je klíčové jak v základním, tak v aplikovaném výzkumu. Naše výsledky významně přispívají k základnímu výzkumu, ale budou mít i praktické důsledky pro ochranářskou biologii druhů.

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Weevils: suitable candidates for prediction of herbivorous insect extinction risk?

Filip Trnka[a], Robert Stejskal[b], Jan Šipoš [c,d] & Tomáš Kuras[a]

[a)] Department of Ecology & Environmental Sciences, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic
[b)] Administration of Podyji National Park, Na Vyhlídce 5, CZ-669 02 Znojmo, Czech Republic
[c)] Department of Vegetation Ecology, Institute of Botany, The Czech Academy of Sciences, Brno, Czech Republic.
[d)] Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel University in Brno, Brno, Czech Republic.

Abstract: One of the most important issues in conservation biology is to reveal and understand the causal mechanisms which determine that particular species will be endangered. We focused on herbivores, the most diverse group of organisms in the world. The application of species life history traits and the ecological traits of their host plants could be good approach to quantify vulnerability more precisely. Until recently, researchers focused only on the life history traits of the studied taxon but not on ecological traits of their host plants. Here we compare life history of endangered and non-endangered species to link the species red-list classification with their life history traits. Phylogenetically corrected analysis allows us to assess the life history and ecological traits leading to population declines and to predict which species could be potentially endangered. We have analyzed the life history traits of nearly 1,000 species of weevils (44% in the Red List) and of their host plants. Our analysis revealed that monophagous, flightless, thermophilic, heliophile, xerophilous and hygrophilous species living in open habitats belong to the most endangered groups among herbivorous weevils. Host plants that prefer xerothermophilous and wetland habitats with low nitrogen content in the soil are mostly linked to the vulnerability of weevils. According to our analysis weevils whose host plants are declining and have low abundance at their biotopes are more vulnerable to the extinction risk and they are connected mainly with aquatic plants or shrubby vegetation. In the complex analysis of all studied characteristics, the life history traits of beetles appear to be more important for predicting threats than the ecological traits of their host plants. However, the traits of weevil host plants are very important and should be used in predictive models together with the herbivore traits.

Key words: conservation, host plants, life history traits, phylogeny, Red List

1. Introduction

Human activities are generally the main causes of the decline of biodiversity in the world (Baillie et al. 2004, Cardinale et al. 2012). Biodiversity is threatened primarily by direct destruction, degradation and fragmentation of habitats (Butchart et al. 2010). There are countless official efforts to prevent biodiversity decline (e.g. CBD 2014), but their success is questionable (Novacek and Cleland 2001, Thomas et al. 2004a, Hooper et al. 2005). The specific causes of species and their populations extinction often remain unknown (e.g. Chapco and Litzenberger 2004) although they are crucial for their appropriate protection and thus for the maintaining of overall biodiversity. Also common species are threatened by the extinction risk, not only the rare or endangered ones (Thomas et al. 2004b, Lindenmayer et al. 2011, Hallmann et al. 2017).

Despite the increasing conservation effort in the last decade, biodiversity is still declining (Kleijn et al. 2011, Sánchez-Bayo and Wyckhuys 2019). Thus there is urgent demand to quantify and predict which characteristic predeterminate species to be endangered in the future. Thus it is in great importance to determine life history traits that are linked with species extinction. All Red Lists based on the IUCN criteria in general are recently studied to predict the extinction risk of different animal species. Furthermore, most of the studies focusing on the predicting extinction risk are devoted mainly to well studied or charismatic groups of invertebrates such as butterflies, dragonflies and damselflies, saproxylic beetles or carabids (cf. Kotiaho et al. 2005, Clausnitzer et al. 2009, Seibold et al. 2015, Nolte et al. 2019). These results are thus only marginally transferable on the other taxons or guilds of arthropod.

Factors that affect extinction risk are not only connected with the particular endangered taxa but also with their environs, especially host plants, concerning herbivores. Herbivorous insect is an important component of the food webs that depict the structure of the ecological communities (Polis and Winemiller 1996). In addition, trophic interaction between consumers and primary producers plays an important role in structuring communities. This highlights how such interaction plays crucial role in the evolutionary and ecological processes shaping species ecological traits and life history. Trophic interactions have been largely ignored in traditional research focusing on extinction risk prediction. The model predicting extinction risk thus should be based also on the ecological traits of the species host plants.

In this study, we have focused on herbivores that are considered as the most diversified group of animals in the world. Weevils are our study group, i.e., beetles of the superfamily Curculionoidea, they belong to the most numerous and most diverse groups of all invertebrates (Oberprieler et al. 2014). This group includes more than 100,000 described species (Astrin et al. 2012) and it is assumed that the real number of species will be around 220,000 or more (Thompson 1992, Oberprieler et al. 2007). Weevils inhabit almost all types of terrestrial and freshwater habitats from arctic zones, high mountains, deserts to tropical forests, where their diversity is maximal (Oberprieler et al. 2014). The larvae and adults of these beetles are mostly phytophagous with negliglible exceptions. Their ability to colonize almost all plants and their different parts (roots, leaves, flowers, fruits and seeds) at different periods of the year gives this group evolutionary advantage. Many weevil species in the Central Europe are considered to be endangered species (e.g. Geiser 1998, Sprick et al. 2003, Benedikt and Strejček 2005). Rarity, threats or local prevalence are often conditioned by strict binding to indigenous natural habitats, respectively specific secondary habitats (e.g. Stejskal and Trnka 2013), but also the rare or local occurrence of their host plants (Mattila et al. 2008).

The decline of biodiversity across wide range of ecosystems is not only due to increasing intensity of disturbance, but also by the abandonment of traditional management in the landscape (coppicing, mowing and pastoral farming) and following loss of the landscape heterogeneity (Benton et al. 2003). Especially European open habitats which are source of diversity (steppe, forest-steppe, pastures and wetlands) are turned to forest-like habitats. It is acknowledged that trophic specialists are more prone to extinction (Kotiaho et al. 2005, Fonseca 2010). Therefore, an accurate prediction of the extinction risk should be based on insect-plant interactions. Changes in open landscape have negatively influenced many threatened species. These species can be characterized by the life history traits that are causes of their population declining.

Therefore, the main objectives of this study are to: i) identify which life history traits indicate extinction risk of the species; ii) describe the relationships between species extinction risk and their life history traits and iii) determine if and how ecological traits of the host plants explain extinction risk of their weevil species.

2. Material and methods

2.1. Phylogeny

Phylogenetic tree branching indicates that species can not appear in statistical analysis as independent values (Felsenstein 1985; Harvey and Pagel 1991). This is because each taxon has evolved from the common lineage and shared evolutionary history which leads to phylogenetic similarity among species (Felsenstein 1985). Reciprocal relationship among species which evolved from mutation and genetic drift depends on phylogenetic distance between species (Harvey and Pagel 1991). Correlation among species data means that linear regression using least squares method cannot be used. Method using generalized least squares (GLS) is appropriate method solving problems with potential heteroskedasticity and autocorrelation of residuals (Garland and Ives 2000). Therefore, we used GLS method to select explanatory variables explaining the largest percentage of deviance in dependent variables.

2.2. Red List classification

Red List of the invertebrates of the Czech Republic (Farkač et al. 2005) was used to assess vulnerability to extinction (independent variable). The degree of the vulnerability of the respective species was classified by categories following the criteria of IUCN. Red List covers all Curculionoidea families. Almost 44% of all species are in the Red List (Benedikt and Strejček 2005).

2.3. Herbivores life history traits

We used the following environmental characteristics: light, heat and moisture requirements of species, ability to fly and the number of host plants (Tab. 1). Data for these traits were obtained from the studies listed in Supporting Information. Some data regarding the habitat requirements have not been published, and in this case, we asked weevil specialists. Analyses of light, temperature and humidity requirements were classified as ordinal variables. For the flying species, we considered those that have wings and that can fly, for the flightless species wings are entirely missing or they have vestigial wings, unable of the flight. Populations of some species may contain both flying and flightless individuals (e.g., *Pseudostyphlus pillumus*, *Hypera contaminata* or *Sitona inops*). We considered these species as flying.

Table 1. List of variables for life history traits of weevils

Variable	Dependent/Explanatory	Unit	Range or definition
Red List	dependent variable	numerical	LC – 0 to RE – 5
Heat requirements	explanatory variable	categorical	thermophilous, mesophilous, indifferent, psychrophilous
Light requirements	explanatory variable	categorical	avoiding light, preferring partial shadow, indifferent, preferring full light
Moisture requirements	explanatory variable	categorical	hydrophilous, mesophilous, indifferent, xerophilous
Flying ability	explanatory variable	categorical	flying, flightless
Degree of polyfagy	explanatory variable	categorical	polyphagous, oligophagous, monophagous

2.4. Ecological traits of host plants

All traits of host plants (Tab. 2.) were based on the study of Ellenberg et al. (1992), only plant life-forms (Raunkiær life-forms) data were based on the study by Jurko (1990) and also traits of some plants because some pannonic species were not included in Ellenberg 's study. Data were classified as ordinal variables. If there was just the host plant genus available from the literature then just one or few plant species from this genus living in the same habitat as the particular weevil were chosen with the botanist. From their values the average was calculated. Complete data are placed in the Supporting Information.

Table 2. List of explanatory variables for ecological traits of weevils host plants.

Variable	**Unit**	**Range or definition**
Raunkiær plant life-form	categorical	A – aquatics (hydrophytes), T – therophytes, H – hemicryptophytes, G – geophytes, C_b – herbaceous chamaephytes C_d – shrubby chamaephytes, F_k – shrubby phanerophytes, F_s – tree phanerophytes
Frequency and endangering	numerical	1 – extremely rare to 9 – nearly everywhere.
Plant flowering	numerical	1 – before spring to 5 – summer.
Tendency of spread	numerical	1 – disappeared or almost disappeared to 6 – rapidly expanding.
Light figure	numerical	1 – plants preferring deep shade to 9 – plants in full light.
Tendency to soil nitrogen	numerical	1 – sites with poor in available nitrogen to 5 – indicator of extremely rich situations.
Tendency to soil moisture	numerical	1 – very dry soils to 5 – wet soils.
Tendency to air temperature	numerical	1 – cold-indicator plant to 5 – indicator of extremely warm conditions.
Plant dominancy	numerical	1 – very isolated to 6 – nearly always dominating or in the large stands.

2.5. Data analysis

The most parsimonious combination of explanatory factors was selected based on lowest Akaike information criterion (AIC) by combining both forward and backward selection (Burnham and Anderson 2002). Generalized linear model (GLM) with Poisson error distribution and log link function was used as null model in stepwise procedure. The most parsimonious model was then refit with generalized least square technique (GLS). . We used GLS method, because it fixes phylogenetic correlation among species using covariance matrix to weight data (Garland and Ives 2000). Phylogenetic covariance matrix was calculated under the Brownian motion model in which variance is accumulated by the time separating species lineages (Martins and Hansen 1997, Revell et al. 2008). We used this type of evolution model because we expect that evolution was random for each species and that particular species trait values could evolve independently on the existing evolutionary history (Revell et al. 2008). Therefore, covariance among species in GLS method was computed by multiplying the variance of Brownian motion and the branch lengths lying between root and the common ancestor (Paradis 2006). For computing branch lengths we created phylogenetic tree for Curculionoidea based on hierarchical taxonomy. The phylogenetic categories used in the analysis were: family, subfamily, genus and species. Data were analysed using the statistical program R (R Development Core Team, 2009).

2.6. Extinction risk prediction: Analytical approach

We used GLM to enable comparison testing by stepwise selection, as well as the estimation of explained variability of the model (R^2 coefficient). Two GLM models were applied: first (Model I) was used to explain degree of vulnerability by using life history traits of weevils and second (Model II) explained degree of vulnerability of weevils by using ecological traits of their host plants. For the Model I we incorporated dataset based on 990 weevil species (also the extinct ones, Supporting Information) that live or have estimated distribution in the Czech Republic or Central Europe includes families Anthribidae, Nemonychidae, Attlabidae, Brentidae, Curculionidae excepting Scolytinae and Platypodinae according to Benedikt et al. (2010). In the Model II analysis 941 weevil species were used whose at least one host plant is known. Ecological traits of host plants were based on Ellenberg et al. (1992) and Jurko

(1990). All traits are given in Tab. 1, 2. In each model degree of vulnerability (base on IUCN criteria) of weevils was used as dependent variable. We then performed stepwise regression combining both forward and backward selection to choose the most parsimonious explanation of the data. In the step-wise regression, each quantitative variable occurred in linear, quadratic and cubic form. Finally, the most parsimonious models (Model I and Model II) were then used to calculate explained variability and were also refit by GLS for correct estimation of model parameters and test statistic.

3. Results

Relevance of life history traits of herbivores and their host plants were tested by two different models (Model I and II). Model I was focused only on life history traits of weevils; using Model II we tested ecological traits of their host plants.

3.1. Herbivores

Using Model I we tested life history traits of the weevils in relation to the risk of the extinction. The best life history traits of weevils were in combination: Heat requirements + Light requirements + Moisture requirements + Flying ability + Degree of polyfagy (Tab. 3). Model I explained 19.63% of the threat of weevils. Analysis revealed heliophile, thermophilic and xerophilic/hygrophilous species connected to open xerothermophilous and/or wetland habitats as the most endangered ones. In addition, monophagous species had a greater risk of extinction than species oligophagous or polyphagous. Mobility of species was also correlated to their extinction risk. Flightless species were vulnerable to extinction more than a flying ones (Fig. 1).

Table 3. Results of the ANOVA of generalized least squares method (GLS) of Model I with phylogenetic correction. Model I explains the vulnerability of weevils by their life history traits. Step-wise procedure was used for choosing explanatory variables for the GLS model. The table shows the most parsimonious combination of explanatory variables explaining the greatest amount of the variability in weevils vulnerability.

	Num DF	**F**	**P**
(Intercept)	1	0.45448	0.5004
Heat requirements	3	33.06246	<0.001
Light requirements	3	11.53104	<0.001
Moisture requirements	3	17.44201	<0.001
Flying ability	1	12.12548	<0.001
Degree of polyfagy	1	40.71046	<0.001

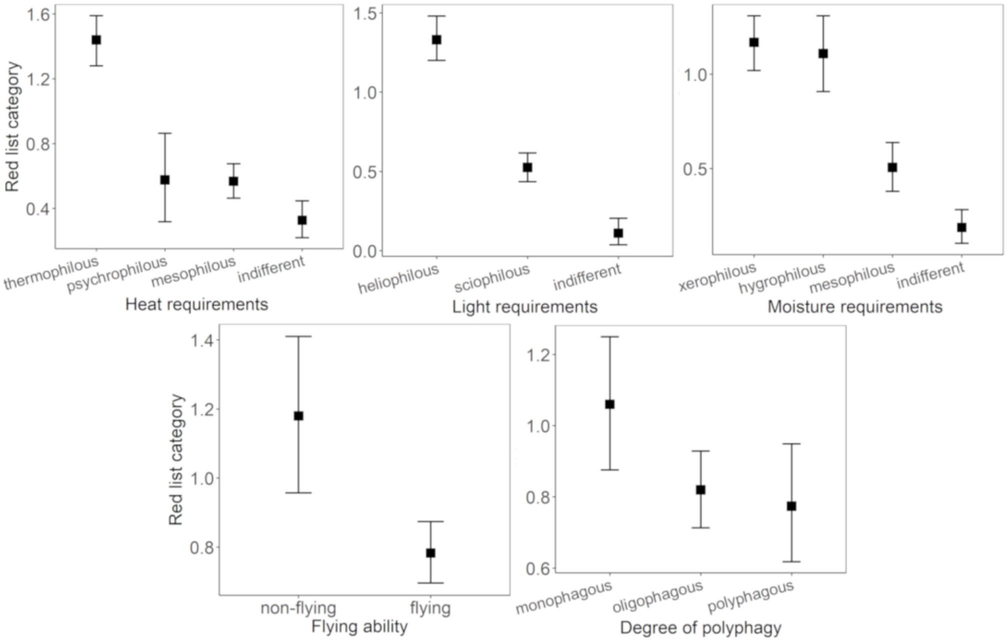

Figure 1. Plots showing the relationship between the heat requirements; light requirements; moisture requirements; flying ability and degree of polyphagy of the weevils and red list category of weevils. Squares represent the mean value and whiskers 95% confidence interval.

3.2. Host plants

In the Model II we tested ecological traits of weevils host plants against extinction risk of weevils. The most parsimonious combinations of environmental traits of host plants were: Tendency to soil moisture + Flowering phenophase + Tendency to spread + Tendency to soil nitrogen + Plant dominancy + Raunkiær plant life-forms (Tab. 4). This combination of variables explained 12.49 % of the threat of weevils based on their host plants. Host plant trait "moisture" explained the most variability of weevil s extinction risk. Host plants preferring dry and wet habitats on the contrary, were correlated with the higher risk of weevil extinction. Plant requirements for nitrogen also clearly explained the higher risk of herbivore extinction. Endangered species of herbivores were more vulnerable at the host plants that prefer a soil with a low content of nitrogen. Higher risk of weevil extinction was connected with the tendency of spread and plant dominancy that means weevils whose host plants were plants that are declining and have low abundance at their habitats were more vulnerable to the extinction risk. Finally, herbivores linked to aquatics and chamaephytes had the higher risk of extinction at all (Fig. 2).

Table 4. Results of the ANOVA of generalized least squares method of Model II with phylogenetic correction. Model II explains the vulnerability of weevils by ecological traits of their host plants. Step-wise procedure was used for choosing explanatory variables for the GLS model. The table shows the most parsimonious combination of explanatory variables explaining the greatest amount of the variability in weevils vulnerability.

	Num DF	**F**	**P**
(Intercept)	1	0.441922	0.5064
Tendency to soil moisture	3	14.785967	<0.001
Plant flowering	1	3.206618	0.0737
Tendency of spread	1	27.008805	<0.001
Tendency to soil nitrogen	1	15.777111	<0.001
Plant dominancy	1	12.138629	<0.001
Raunkiær plant life-form	1	28.592239	<0.001

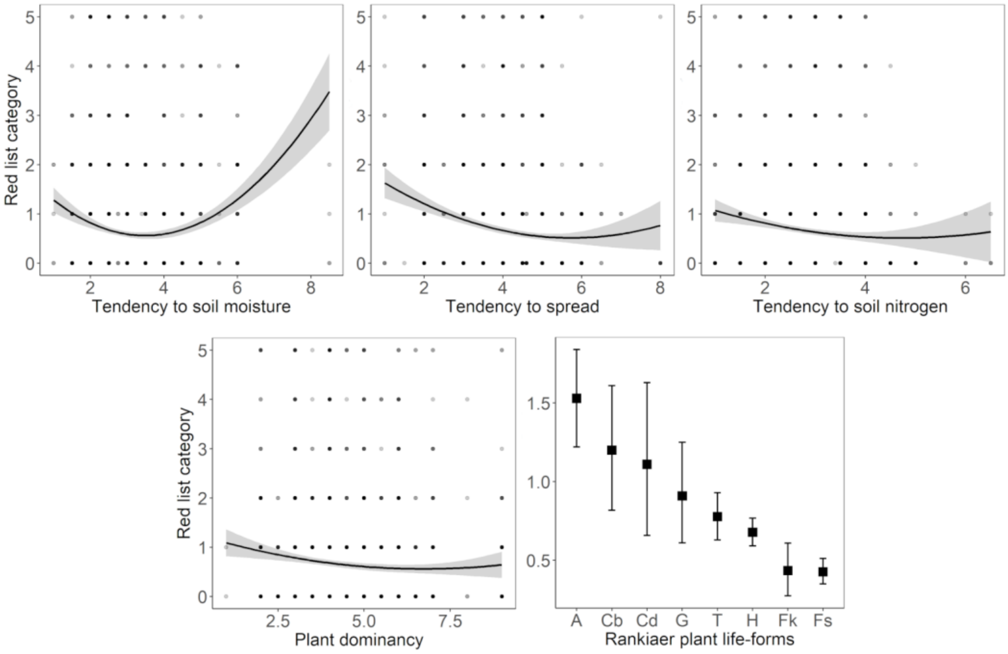

Figure 2. Scatter plots showing the relationship between the tendency to soil moisture of the plants; flowering phenophase; tendency of change; tendendy to soil nitrogen; plant dominancy; Raunkiær plant life-form (aquatics as hydrophytes, T – therophytes, H – hemicryptophytes, G – geophytes, Cb – herbaceous chamaephytes Cd – shrubby chamaephytes, Fk – shrubby phanerophytes, Fs – tree phanerophytes) and red list category of weevils. Regression curve with marked 95% confidence intervals were fitted by the generalized liner model with Gaussian error distribution and link function "identity". Explanatory variable of the model was set as a second-degree polynomial.

4. Discussion

Most studies focusing on the extinction risk predictions incorporate only life history traits of the herbivores and do not include the ecological traits of their host plants (e.g. Kotiaho et al. 2005, Mattila et al. 2006, Mattila et al. 2008, Pavlikova and Konvicka 2012, Potocký et al. 2018), This may leads to: i) misidentification of the key factors causing the extinction risk; ii) misinterpretation causes of the species vulnerability; and iii) biased prediction of the red-list statut (Nylin and Bergstrëm 2009). Therefore, we assumed that integrating ecological traits of host plants is important to improve the accuracy and reliability of the prediction of actual herbivore extinction risk.

4.1. Life history traits of herbivores

According to our results, factors directly related to the life history traits of herbivores better estimate their risk of extinction than factors related only to the host plant. Based on our results we can state that the most endangered individuals are monophagous species with low dispersal ability and that inhabit open and xerothermic or waterlogged habitats. Similar conclusions were found for butterflies and moths (Kotiaho et al. 2005, Mattila et al. 2006). Monophagous herbivore species are confirmed to be more susceptible to extinction, because they have a narrow food niche and often reduced ability to spread (Mattila et al. 2008, Friedmann 2009). Especially in the case of flightless species with limited distribution, they are at risk of isolation and fragmentation of habitats. Due to the fragmentation of habitats species have a reduced chance to re-colonize suitable habitats (Zera and Denno 1997, Thomas et al. 2001, Buse 2012).

Correlation between ecological traits and species vulnerability indicate that the high extinction risk of weevils is associated with thermophilous, heliophilous and xerothermous habitats, inhabited by: e.g. subfamily Lixinae, genera *Baris*, *Hypera*, *Minyops*, *Mogulones* or *Trachyphloeus*. The same link between species vulnerability and habitat types was reported by Sprick et al. (2003) or Stejskal and Trnka (2012, 2013). According to these studies higher percentage of the endangered weevils were recorded in groups of heliophilous and thermophilous species. These habitats (i.e. steppes, sands, alpine grasslands, rocks, bogs) can include “man-made” secondary habitats like dry grasslands, meadows, pastures, fallows, field margins, groves, coppice woods etc. (van Helsdingen et al. 1996). These types of habitats are declining nowadays in European landscape (Blaxter and Robertson 1995). Therefore, the greatest threat for these species is decline of traditional methods of landscape managements (i.e., coppicing, grazing, woodland pasture, pastoral farming, mowing of meadows) (Vera 2000, Benton et al. 2003, Horak et al. 2014) or use of synthetic pesticides on these habitats (Dudley and Alexander 2017).

High degree of extinction risk was not only predicted for herbivores living in dry and hot habitats, but also for hydrophilous herbivores (e.g. *Apoderus erytropterus*, *Bagoopsis globicollis*, *Bagous* spp., *Chlorophanus* spp., *Hypera arundinis*, *Icaris sparganii*, *Lixus paraplecticus*, *Poophagus hopffgarteni* or *Sphenophorus abbreviatus*). The risk of extinction of herbivores feeding on wetland plants is given by their low dispersal ability, host plant specialization and high sensitivity to environmental pollution (Sprick 2003). Gradual pollution, changes in water regime and destruction of littoral of aquatic habitats lead to rapid disappearance of these species (Strejček 2001, Sprick 2003).

Another main determinant of extinction risk predicted by our model was geografical distribution of the species (Cowley et al. 2001, Päivinen et al. 2005). It is assumed that suitable patches on the edge of species distribution are more isolated than those in core localities (Päivinen et al. 2005). Populations at the range edge alone lead to significantly lower population sizes and they are more stenotopic on habitats and host plants in the periphery of the range than in the core (Bahn et al. 2006, Fourcade and Öckinger 2017). Lower population sizes mean higher extinction risks (Bourn and Thomas 2002, Berggren 2009). Some species are naturally rare and Central Europe is the edge of their natural area such as the Mediterranean and Pontic region (e.g. Benedikt et al. 2010). Thus there is a high risk of their extinction due to the very specific demands on habitats and host plants (e.g. *Apsis albolineata*, *Bothynoderes declivis* or *Brachycerus foveicollis*).

4.2. Ecological traits of host plants

The proportion of explained variance in model using only ecological traits of host plants (12.49%) was lower than in the model using only ecological traits of herbivores (19.63%). Moisture requirements of the host plants best explain the risk of extinction of herbivores. This fact correlates strictly with life history traits of herbivores themselves, which belongs to endangered species if they are linked to dry or wetland habitats. Correlation of host plants with dry and sunny habitats shows that herbivores living on these plants are threatened by extinction the most. Similar results can be found in the study of Sprick et al. (2003), who showed that majority of endangered weevils lives in open habitats like steppe grasslands and sands where heliophilous and xerothermophilous plants grow. A large number of specialist species is tied to these habitats (Willems et al. 1993). Declining abundance of these species is probably caused by the abandonment of traditional landscape management, as mentioned above.

Our results show that herbivores with a link to the open habitats have a greater risk of extinction than species with connection to forest habitats. According to our analysis, endangered weevil species are also connected to the host plants that prefer nitrogen-poor soils. Such habitats include steppe grasslands, sands, salt marshes or peat bogs. Plant species adapted to nutrient-poor soils suffered highest rates of extinction in all habitat types also according to Stehlik et al. (2007). Vegetation overgrowing of these habitats accumulates humus, which puts relict species to the disadvantage because they require only a thin layer of heated dried humus, otherwise they recede these habitats (Strejček 2001). Eutrophication affects the heat, light and moisture conditions of habitat. Increase of the availability of nitrogen causes shifts in species composition, overall reduction of biodiversity and changes in the structure of food webs in ecosystems (Erisman et al. 2013). Also the ecological traits tendency of spread and plant dominancy were connected with the higher extinction risk of weevils. Host plants that have tendency to decline or have low abundances on habitats were more vulnerable. It is well known that the distribution of host plants limits the distribution of species living at these plants (Mattila et al. 2008). The connection of weevils with aquatics and chamaephytes plant life-forms caused that they are threatened by extinction risk more. In Central Europe aquatics and chamaephytes were the least frequent forms of plants (Kaplan 2012). Aquatics were prone to be vulnerable to the extinction risk the most of all of the plant life-forms (Stehlik et al. 2007). Regarding chamaephytes, for example genus *Calluna*, *Cytisus*, *Genista* or *Helianthemum* were typical for open or semi-open habitats and only these four genera were the host plants for 31 weevil species of which 16 ones are in Red List (cf. Supporting Information).

4.3. Conclusions

The models showed that the life history traits of the studied herbivores prevailed influence of the ecological traits of their host plants in predicting of their extinction risk. The percentage of explained variability in weevil vulnerability explained only by host plants ecological traits was not negligible. Therefore, information about the host plants should be included in the prediction models assessing the risks of extinction and threats to herbivores.

Generally, the analysis revealed that the potentially endangered species include those that live in open habitats and are monophagous and flightless. These are mainly open xerothermophilous communities and wetland habitats. Weevils whose host plants are plants that are declining and have low abundances at their habitats were more vulnerable to the extinction risk. Within these habitats endangered weevils were linked to aquatics or chamaephytes (shrubby vegetation). Threats to these habitats and their host plants probably lie in the fragmentation, degradation and direct loss of these habitats, but also in abandoning the traditional type of management, such as grazing, mowing or burning.

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PŘÍLOHA II

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Biology and morphology of immature stages of *Coniocleonus nigrosuturatus* (Coleoptera: Curculionidae: Lixinae)

Robert STEJSKAL[1], Filip TRNKA[2] & Jiří SKUHROVEC[3]

[1] Department of Forest Botany, Dendrology and Geobiocoenology, Mendel University in Brno, Zemědělská 1, CZ-613 00 Brno, & Administration of Podyji National Park, Na Vyhlídce 5, CZ-669 02 Znojmo, Czech Republic; e-mail: rstejskal@centrum.cz

[2] Department of Ecology & Environmental Sciences, Faculty of Science, Palacký University Olomouc, Tř. Svobody 26, CZ-771 46 Olomouc, Czech Republic; e-mail: filip.trnka88@gmail.com

[3] Group Function of invertebrate and plant biodiversity in agro-ecosystems, Crop Research Institute, Drnovská 507, CZ-161 06 Praha 6 – Ruzyně, Czech Republic; e-mail: jirislavskuhrovec@gmail.com

Abstract. Mature larvae and pupae of *Coniocleonus* (*Plagiographus*) *nigrosuturatus* (Goeze, 1777) (Curculionidae: Lixinae: Cleonini) are described and compared with three other cleonine taxa with known larvae. The biology of the species was studied in Romania, Hungary and Slovakia. Common Stork's-bill (*Erodium cicutarium*) (Geraniaceae) is identified as a host plant of both larvae and adults of this weevil. The weevil is very likely monophagous, and previous records of thyme (*Thymus* sp., Lamiaceae) as the host plant hence appear incorrect. *Coniocleonus nigrosuturatus* prefers dry, sunny places in grassland habitats, with sparse vegetation, bare ground and patchily growing host plants. Overwintering beetles emerge in early spring (March), feed and mate on the host plants. The highest activity of adults was observed from mid-April to mid-May. Larvae live in the soil in a vertical tunnel near the host plant and feed externally on the base of the stem. Usually, a single larva was observed at an attacked plant, however, sometimes up to 3 larvae were found. At the beginning of July, the larvae pupate in a closed earthen cell. A reared adult emerged in the laboratory at the end of July. We suppose that part of the population overwinters in pupation cells, although some adults leave the cells at the end of summer. They then most likely spend some time feeding on the host plants and look for suitable shelters in which to overwinter later.

Key words. Coleoptera, Curculionidae, Lixinae, *Coniocleonus nigrosuturatus*, mature larva, pupa, morphology, host plant, *Erodium cicutarium*, Geraniaceae, larval development, life history, central Europe, Palaearctic Region

Introduction

Weevils of the subfamily Lixinae (Coleoptera: Curculionidae) are medium to large-sized beetles distributed mainly in Palaearctic and Afrotropic Regions. The subfamily includes approximately 1,200 species (Ter-Minasian 1967). Although generally accepted as a separate subfamily (e.g., Löbl & Smetana 2013), the group is occasionally treated as a part of the Molytinae (Oberprieler et al. 2007) or Curculioninae (Lawrence & Newton 1995). Lixinae is considered a rather derived weevil group standing close to the Hyperinae Marseul, 1863 and Mesoptiliinae Lacordaire, 1863, and is divided into two tribes: Cleonini Schönherr, 1826 and Lixini Schönherr, 1823 (Löbl & Smetana 2013). Although a preliminary phylogenetic analysis is only available for Mediterranean and Afrotropical taxa, a general analysis of the whole tribe is now in progress (Meregalli & Silvestro 2008, unpubl. data).

Until recently, the immature stages of the Lixinae were largely unknown and unstudied (Anderson 1987). The situation has slightly changed within the last 10 years, but this mainly concerns the tribe Lixini, for which several studies describing the morphology of immature stages have been published (Nikulina 2001, 2007; Nikulina & Gültekin 2011; Gosik & Skuhrovec 2011; Gosik & Wanat 2014). The knowledge of the immature stages of the Cleonini remains very limited, as immature stages of only three species have been described by Scherf (1964).

The genus *Coniocleonus* Motschulsky, 1860, belongs to the tribe Cleonini. It comprises 27 known species classified in five subgenera (Meregalli & Fremuth 2013). All species are known from the Palaearctic Region, two of them also reach the Nearctic Region. Seventeen species are known from Europe, occurring mainly in the Mediterranean area (Meregalli & Fremuth 2013).

Data on the biology of the species of *Coniocleonus* are very scarce. They prefer warm and dry habitats in steppe or semidesert regions (Ter-Minasian 1967, 1988; Dieckmann 1983; Koch 1992). Host plants are unknown for the majority of species. For a few species, only data on refuge plants are available, which are used as shelter for adults and in some cases may be food sources. *Coniocleonus cicatricosus* (Hoppe, 1795) and *C. nebulosus* (Linnaeus, 1758) were collected under stands of *Calluna vulgaris* or *Erica* spp. (Ericaceae) (Koch 1992). *Coniocleonus hollbergi* (Fåhraeus, 1842) and *C. turbatus* (Fåhraeus, 1842), both of which occasionally feed on some woody plants (e.g., *Pinus sylvestris*, *Quercus* spp.), are supposed to develop on *Rumex acetosella* (Polygonaceae) (Dieckmann 1983, Bayer & Winkelmann 2005, Rheinheimer & Hassler 2010).

Larval development has been observed only for two species: *Coniocleonus astragali* Ter-Minasian & Korotyaev, 1977, which developed on the roots of *Astragalus* sp. and *Oxytropis* sp. (both Fabaceae) with larvae and pupae hidden in chambers made of plant debris and excrements (Ter-Minasian 1988). Ectophagous larvae are also known for *Coniocleonus excoriatus* (Gyllenhal, 1834), which is associated with *Emex spinosa* (Polygonaceae) (Julien 1980, Scott & Yeoh 1996). Nevertheless, descriptions of the immature stages of these weevils have not been published.

One of the species with very poorly known biology is *Coniocleonus nigrosuturatus*. The species belongs to the subgenus *Plagiographus* Chevrolat, 1869 (Meregalli & Fremuth 2013) which is regarded as a separate genus by some authors (e.g., Arzanov 2006). The species' range includes North Africa, a large part of Asia to western Siberia and most of Europe except the northern part and British Isles (Meregalli & Fremuth 2013). In central Europe, the species is regarded as a xerothermic stenotopic species inhabiting sandy habitats, heathlands, dry grasslands and pastures (Koch 1992). In several central European countries, *C. nigrosuturatus* is an endangered species (e.g., Geiser 1998, Benedikt & Strejček 2005, Stejskal & Trnka 2013), and this species is only known from historical records in many regions (Lohse 1983). Thus far, only thyme (*Thymus* sp.) was reported as host plant of adults, without mentioning other details of the biology of the species, likely first reported by Kleine (1910). Later authors either only repeated his data (Fleischer 1927–1930, Fremuth 1982, Ter-Minasian 1988, Arzanov 2006) or regarded *C. nigrosuturatus* as a species with an unknown host plant (e.g., Dieckmann 1983, Lohse 1983). Thérond (1976) listed the findings of *C. nigrosuturatus* on *Limonium* sp. (Plumbaginaceae) in France. The immature stages of *C. nigrosuturatus* have never been observed. In this paper, we describe immature stages of the species and provide details on its life history based on our observations obtained during field work in Romania, Hungary and Slovakia.

Material and methods

The material used to describe immature stages was collected, and field observations were conducted, in the following localities:

ROMANIA: **Caraș-Severin County:** Sfânta Elena env., 44°40′42″N, 21°43′14″E. Visit dates: 26.iv.–1.v.2010, 28.iv.–1.v.2011, 24.–28.vii.2011, 4–9.vi.2012, 18.–24.v.2013, 4.vii.2013. Habitats: pastures (cow, sheep, goats), road margins and dry grasslands. Bedrock: limestone. Altitude: 400 m a. s. l. (see Figs 5D–E).

HUNGARY: **Bács-Kiskun County:** Petőfiszállás env., 46°36′33″N, 19°50′19″E. Visit dates: 18.v.2013. Habitat: closely cut lawn around a petrol station. Bedrock: sand. Altitude: 90 m a. s. l.

SLOVAKIA: **Trnava Region:** Dolné Zelenice env., 48°22′6″N, 17°45′2″E. Visit dates: 9.v.2013 and 16.vi.2013. Habitats: dry grasslands and road margins. Bedrock: alluvial sediments (sandy gravel). Altitude: 130 m a. s. l. **Nitra Region:** Kolíňany env., 48°20′35″N 18°11′15″E. Visit dates: 26.v.2012. Habitats: military area with disturbed grasslands and free sand. Bedrock: tertiary deposits (loess), sand. Altitude: 300 m a. s. l. (see Fig. 5F).

Rearing and life cycle observations were conducted during the vegetation seasons of 2012–2013. Laboratory observations were conducted in Sfânta Elena, Romania, in Znojmo, Czech Republic (48°51’31”N, 16°2’40”E) and in Olomouc, Czech Republic (49°35′36″N, 17°15′3″E) using larvae from Sfânta Elena, Romania, and Dolné Zelenice, Slovakia. Host plant associations were studied by direct observation in the field and by testing selected plants in the laboratory.

Larvae were collected by R. Stejskal and F. Trnka in the soil within the vicinity of the host plant (*Erodium cicutarium*) and maintained in the laboratory using the following two rearing techniques: (1) Some larvae were excavated using a garden trowel along with the complete host plant including its root ball and soil. The plant was then cultivated in a common flowerpot. (2) Some larvae were first removed from their soil tunnels and then placed in a prepared flowerpot with the host plant. In these cases, the host plants were cultivated in soil sifted in advance to remove unwanted materials and organisms (e.g., carnivorous beetle larvae).

The flowerpots containing the host plants and larvae were placed in a sunny place at room temperature and moistened approximately once every 3–5 days. Moulding or dead plants were replaced with new plants.

Part of the material of the larvae and pupae was preserved in Pampel fixation liquid (4 parts glacial acetic acid, 6 parts 4% formaldehyde, 15 parts 95% ethylalcohol and 30 parts distilled water) and used for the morphological descriptions. These specimens are now deposited in the Group Function of invertebrate and plant biodiversity in agro-ecosystems of the Crop Research Institute (Prague, Czech Republic). Plants were identified by the collectors and the identification was reviewed by a botanist.

Slides were prepared as follows (for details see May 1994): a larva was decapitated, and its head was rinsed in distilled water and cleaned in a 10% solution of potassium hydroxide (KOH). Then, the mouthparts were separated from the head capsule. All body parts were then mounted on temporary microscope slides in 10% glycerin.

The specimens were examined and measurements were made using a light microscope with ocular scale (Olympus SZ60 and SZX16). The following characteristics were measured for each larva (head width; length of the body [larvae fixed in C-shape were measured by segment], width of the body in the widest place [abdominal segments]) and pupa (length and width). The thorax and abdomen are not sclerotised and their proportions may be affected by the fixation process, thus measurements of these parts are only given for comparison purposes.

Drawings were made using a drawing tube attached to the light microscope and processed by computer (GIMP 2). The thoracic spiracle is placed on the boundary of the prothorax and mesothorax, as shown in the drawings (see Fig. 2B); however, it is in fact of mesothoracic origin (Marvaldi et al. 2002, Marvaldi 2003). Drawings of the thoracic and abdominal spiracles are schematic (see Figs. 2B–D). Terms and abbreviations regarding the setae of the mature larva and pupa follow Scherf (1964), May (1977, 1994) and Marvaldi (1998).

Morphology of mature larva and pupa

Coniocleonus nigrosuturatus (Goeze, 1777)

Material examined. ROMANIA: Sfânta Elena: 24.v.2013 (1 larva), 4.vii.2013 (1 larva, 1 pupa) (all F. Trnka leg.), 7.vi.2012 (1 larva), 10.vii.2012 (1 pupa reared, partly damaged), 19.v.2013 (2 larvae), 7.vii.2013 (1 pupa reared from larva from v.2013 (partly dried-up)) (all R. Stejskal leg.). **SLOVAKIA:** Dolné Zelenice, 16.vi.2013 (2 larvae), R. Stejskal leg.

Description of mature larva. ***Colouration.*** Head light brown or brown. All thoracic and abdominal segments white (Fig. 4A). Cuticle finely spiculate.

Head capsule and mouth parts. Head width: 1.5–1.7 mm (mean 1.6 mm), suboval, flattened laterally, endocarinal line absent. Frontal sutures on head distinct, Y-shaped, extended to stemmata. Single stemma (st), in the form of a dark pigmented spot, located on each side anterolaterlly. *Des1* near to frontal suture, *des2* and *des3* located in the central part of epicranium, *des4* located anterially near to frontal suture, *des5* located anterolaterally; *des1-2* and *des4-5* long, equal in length, *des3* distinctly shorter (Fig. 1A). *Fs1* and *fs2* placed medially, *fs3* located anteriomedially, *fs4* located anteriolaterally, and *fs5* located laterally, close to the epistoma; all setae relatively long, *fs3* and *fs4* shorter than the remaining three setae (Fig. 1A). *Les1–2* as long as *des1*; and *ves1–2* as long as *des3*. Epicranial area without pores. Antennae

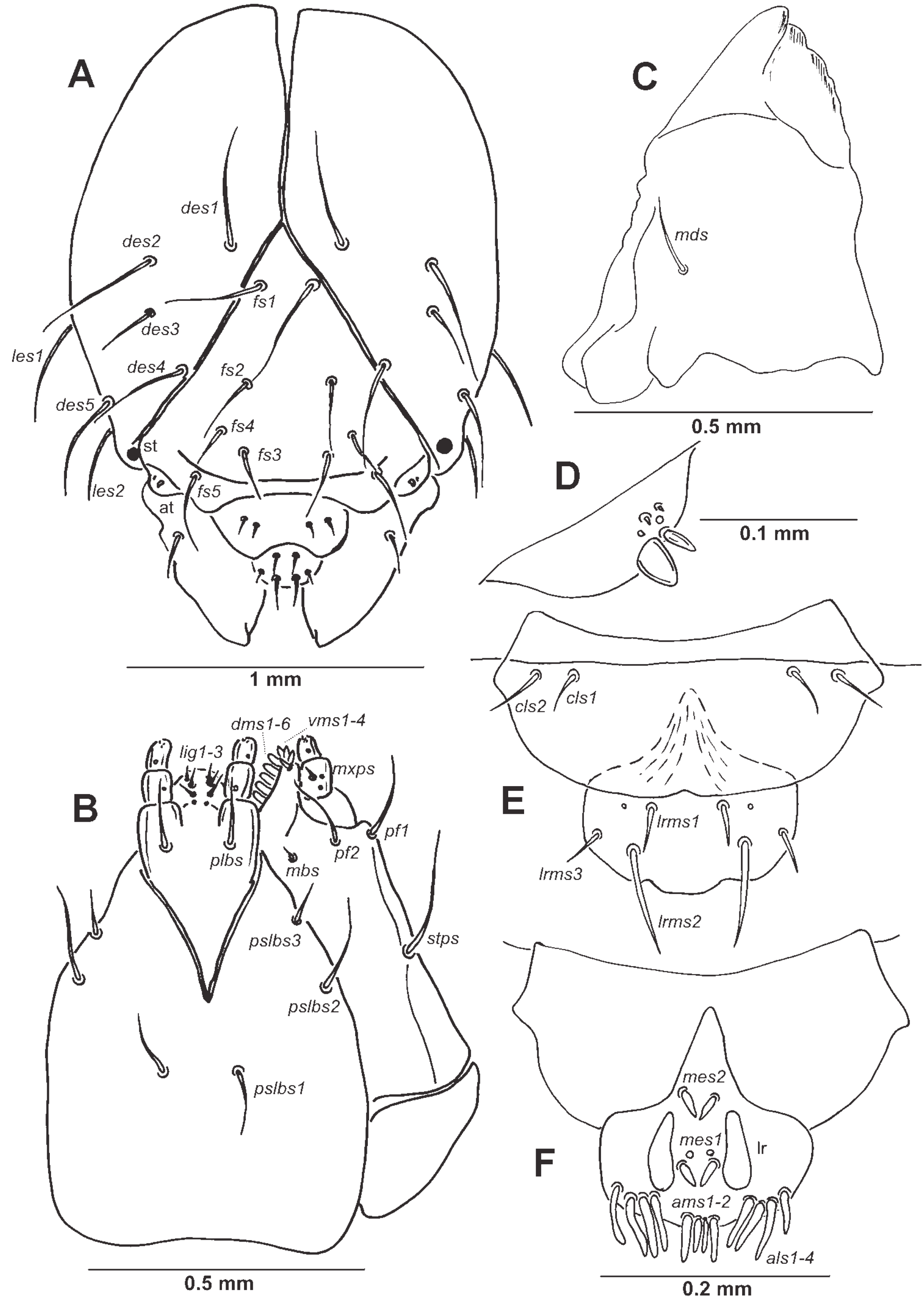

Fig. 1. *Coniocleonus nigrosuturatus* (Goeze, 1777), mature larva head, antenna and mouth parts: A – dorsal view (*des* – dorsal epicranial seta(e), *fs* – frontal s., *les* – lateral epicranial s., st – stemmata, at – antenna); B – right maxilla and labium (both ventral view) (*dms* – dorsal malae s., *vms* – ventral malae s., *mpxs* – maxillary palps s., *mbs* – basiventral s., *pfs* – palpiferal s., *stps* – stipal s., *plbs* – prelabial s., *pslbs* – postlabial s., *ligs* – ligular s.); C – right mandible (*mds* – mandible dorsal s.); D – antenna; E – labrum and clypeus (*cls* – clypeal s., *lrms* – labral s.); F – epipharynx (*ams* – anteromedial s., *als* – anteriolateral s., *mes* – median s., lr – labral rods).

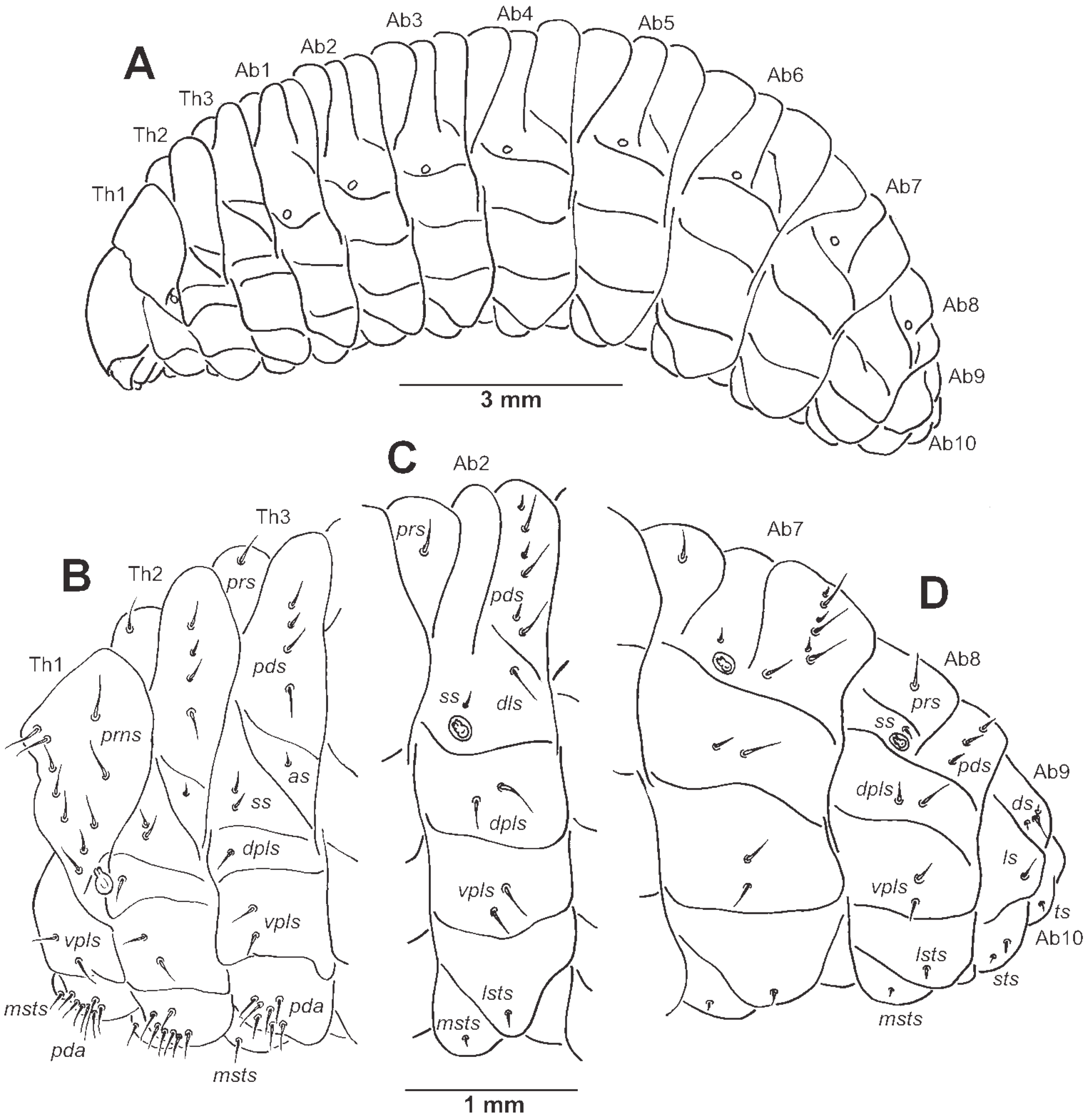

Fig. 2. *Coniocleonus nigrosuturatus* (Goeze, 1777), mature larva habitus: A – shape of the body (lateral view), B – lateral view of thoracic segments, C – lateral view of abdominal segment II., D – lateral view of abdominal segments VII–X (*prns* – pronotal seta(e), *prs* – prodorsal s., *pds* – postdorsal s., *as* – alar s., *ss* – spiracular s., *dpls* – dorsopleural s., *vpls* – ventropleural s., *pda* – pedal s., *dls* – dorsolateral s., *lsts* – laterosternal s., *msts* – mesosternal s., *ds* – dorsal s., *ls* – lateral s., *sts* – sternal s., *ts* – terminal s.; Th1-3, Ab1-10 – number of thoracic or abdominal segment).

located at the end of the frontal suture on each side, membranous and slightly convex basal article bearing conical triangular sensorium, relatively long; basal membranous article with 3 sensillae different in both shape and length (Fig. 1D).

Labrum (Fig. 1E) approximately 2 times as wide as long, with 3 pairs of hairform *lrms*, of different length; *lrms3* and *lrms1* distinctly shorter than *lrms2*; *lrms1* placed close to the margin with clypeus, *lrms2* located anteriomedially and *lrms3* located anteriolaterally; anterior margin double sinuate. Clypeus (Fig. 1E) approx. 2.5 times as wide as long with 2 relatively short *cls*, unequal in length, localized posteriolaterally; anterior margin rounded to the inside; median part covered by thorn-shaped cuticular processes. Epipharynx (Fig. 1F) with 4 pairs of blunt, finger-like *als*, of almost equal length; 2 pairs of *ams*, *ams1* distinctly shorter than *ams2*; 2 pairs of short, blunt *mes*; labral rods (lr) elongated, converging anterially. Mandibles (Fig. 1C) relatively broad, bifid, tooth of unequal height; slightly truncate; *mds* relatively long, hairform. Maxilla (Fig. 1B) stipes with 1 *stps*, 2 *pfs* and 1 *mbs*, *stps* and *pfs1-2* long, equal in length, *mbs* very short; mala with 6 bacilliform *dms* of different length; 4 *vms* short, almost equal in length; *vms* distinctly shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with 1 *mxps* and two pores; length ratio of basal and distal palpomeres: 1:0.7; distal palpomere with one pore and a group of conical, cuticular apical processes. Praelabium (Fig. 1B) heart-shaped and distinctly elongated, with 1 pair of *plbs*; ligula with sinuate margin and 3 pairs of hairform micro *ligs*, equal in length; premental sclerite well visible. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres: 1:0.6; distal palpomere with one pore and short, cuticular apical processes; basal palpomere with 1 dorsal pore. Postlabium (Fig. 1B) with 3 *pslbs*, *pslbs1* located anterially, remaining two pairs laterally; different in length, *pslbs3* distinctly shorter than *pslbs1* and *pslbs2*.

Thorax and abdomen. Body length: 11.0–13.5 mm (mean 12.0 mm) stocky, slightly curved, rounded in cross section (Fig. 2A). The widest place in the body (abdominal segments V–VI) measuring up to 4.5 mm. Prothorax distinctly smaller than meso- and metathorax. Metathorax and abdominal segments I–III of almost equal length, next abdominal segments increasing gradually to abdominal segments V–VI (the largest) and then decreasing to the terminal parts of the body. Abdominal segment X reduced to four anal lobes of unequal size, the dorsal being distinctly the largest, the lateral pair equal in size, and the ventral lobe very small. Anus located terminally. Spiracles (9 pairs) bicameral, the first placed between the pro- and mesothorax (see Material and methods), the abdominal spiracles located laterally, close to the anterior margin of abdominal segments I–VIII.

Chaetotaxy of mature larva. Slightly reduced, but in some parts with more than the most frequent number of setae in weevils (e.g., meso- and metathorax with 2(3) *pds*, and abdominal segments I-VII with 5 *pds*; the state in *C. nigrosuturatus* see below). Setae thin, long, light yellow or orange. ***Thorax.*** Prothorax (Fig. 2B) with 10 *prns* unequal in length, 2 *vpls* and 1 *msts*. Mesothorax (Fig. 2B) with 1 *prs*, 4 *pds* (*pds2* distinctly shorter than the three remaining setae), 1 very short *as*, 2 short *ss*, 1 *dpls*, 2 *vpls* of different length and 1 short *msts*. Chaetotaxy of metathorax (Fig. 2B) identical to mesothoracal. Each pedal area of thoracic segments well separated, with 6-8 *pda* unequal in length. ***Abdomen.*** Abdominal segments I-VII (Figs 2C–D) with 1 *prs*, 6 *pds* (*pds1*, *pds3* and *pds5* very short), 1 *dls*, 1 very short *ss*, 2 *dpls* of different length, 2 *vpls*, 1 very short *lsts* and 1 micro *msts*. Abdominal segment VIII (Fig. 2D) with 1 *prs*, 3 very short *pds*, 1 very short *ss*, 2 *dpls* of different length, 2 short *vpls*, 1 very short *lsts* and 1 micro *msts*. Abdominal segment IX (Fig. 2D) with 3 *ds* (*ds2* relatively long, *ds1* and *ds3* microsetae); 1 short *ls* and 2 *sts* of different length (*sts1* very short, *sts2* microsetae). Each anal lobe on abdominal segment X (Fig. 2D) with 1 microseta (*ts*).

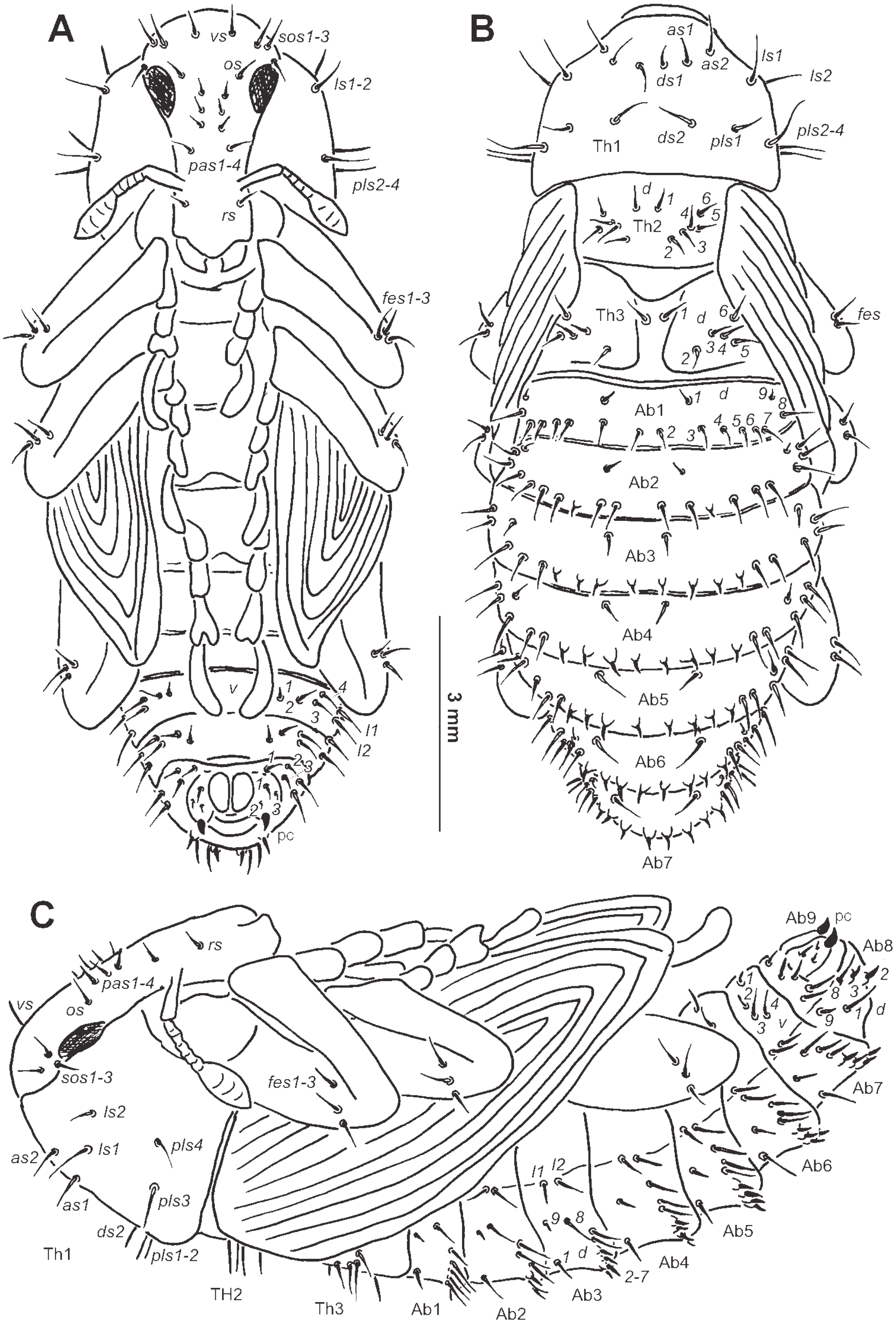

Fig. 3. *Coniocleonus nigrosuturatus* (Goeze, 1777), pupa habitus: A – ventral view, B – dorsal view, C – lateral view (*vs* – vertical seta(e), *sos* – superorbital s., *os* – orbital s., *pas* – postantennal s., *rs* – rostral s., *as* – apical s., *ls* – lateral s., *ds* – discal s., *pls* – posterolateral s., *fes* – femoral s., *d* – dorsal s., *l* – lateral s., *v* – ventral s.; Th1-3, Ab1-10 – number of thotacic and abdominal segment; pc – pseudocerci).

Description of pupa. ***Morphology*** (Figs 3A–C, 4C). Body length: 11.0–11.6 mm, at the widest region: 4.5–5.3 mm. The widest place in the body is commonly between the apex of the meso- or metafemora. Body stocky, elongated, white or yellowish. Cuticle smooth. Rostrum relatively short, approximately 1.8 times as long as wide, extended beyond procoxae. Antennae relatively long and stout. Pronotum almost 1.7 times as wide as long. Mesonotum and metanotum of almost equal length. Abdominal segments I–V of almost equal length; abdominal segment VI semicircular, next abdominal segments diminish gradually to the end of the body. Abdominal segments VII–IX distinctly smaller than other abdominal segments. Gonotheca (abdominal segment IX) of all three specimens divided. Sexual dimorphism in weevils is visible mainly in the length of rostrum and in the structure of abdominal segment IX: gonotheca of ♂ undivided, of ♀ divided (Gosik & Sprick 2012a,b, 2013; Gosik & Wanat 2014).

Chaetotaxy (Figs 3A–C). Setae relatively long, unequal in length, light yellow or orange, some setae on abdominal segments II–VIII distinctly get stronger and located on protuberances. Setae well visible. Head capsule includes 1 *vs*, 3 *sos*, 1 *os* and 4 *pas*. Rostrum with 1 *rs*, located on the anterior margin. Setae on head capsule and rostrum straight, *rs* and *pas1–3* distinctly shorter than the remaining setae on head, thoracic and abdominal segments.

Pronotum with 2 *as*, 2 *ds*, 2 *ls* and 4 *pls*. Dorsal parts of mesothorax with 1 pair of setae located posteromedially and 5 pairs located along its anterior margin. Chaetotaxy of metathorax identical to mesothoracal. Each apex of femora with groups of 3 *fes*. Dorsal parts of abdominal segments I–VIII each with 2 pairs of setae located posteriorly (*d1*, *d9*) and 7 pairs (*d2-8*) located along theirs anterior margins. Seta *d4* (on abdominal segment II) and setae *d2-5* (on abdominal segments III–VII) short, thorn-like, located on protuberances. Remaining setae long, hair-like. Abdominal segments I–VII with groups of 2 lateral setae and 4 pairs of ventral setae. Dorsal part of abdominal segment VIII with 2 pairs of setae located posteriorlly (*d1*, *d9*) and 3 pairs (*d2, d3* and *d8*) located along its anterior margin; *d2-–3* thorn-like, located on protuberances; remaining setae elongated. Abdominal segment VIII with groups of 2 lateral setae and 3 pairs of short ventral setae. Abdominal segment IX with 2 pairs of ventral microsetae and 1 pair of short, thin setae. Pseudocerci short, triangular.

Comparison with larvae of other Cleonini. Larvae of three cleonine taxa have been described so far (Scherf 1964): *Cleonis pigra* (Scopoli, 1763), *Cyphocleonus dealbatus* (Gmelin, 1790) (as *Cyphocleonus tigrinus* (Panzer, 1789)), and ?*Pachycerus segnis* (Germar, 1824) (as *Pachycerus scabrosus* Brullé, 1832, but identification is probaly incorrect, see below). The comparison of the larva of *Coniocleonus nigrosuturatus* with those decribed by Scherf (1964) is somewhat problematic due to the use of differing terminology for morphology and chaetotaxy and/or an absence of good quality drawings. Despite these problems, we were able to compare the morphology of all four taxa (Table 1). However, the identity of the larva described as *Pachycerus segnis* by Scherf (1964) is unclear and it likely represents a genus other than *Pachycerus.* According to extensive surveys by Brun et al. (1993) conducted in France, Greece and Turkey, larvae of *P. segnis* were always found within earthen cells attached to the taproot, but not in gall-like swellings as reported by Scherf (1964). Descriptions by Scherf (1964) perfectly fit the larval development of some species of the cleonine genus

Table 1. Differential diagnosis of mature larvae and pupae of 4 species from the tribe Cleonini.

	Coniocleonus nigrosuturatus	***Cleonis nigra***	***Cyphocleonus dealbatus***	**'*Pachycerus scabrosus*' sensu SCHERF (1964)***
Larva				
Endocarina	absent	present	absent	absent
Number of stemmata	1	2	1	1
Number of *des*	5	4	5	5
Number of *fs*	5	4	5	5
Number of *les*	2	not presented	3	3
Number of *ves*	2	not presented	not presented	not presented
Number of *cls*	2	2	2	3
Position of *lrms1–3*	in a triangle	in a triangle	in a triangle	in a line
Number of *als*	4	4	6	3
Number of *mds*	1	2	2	2
Number of *ligs*	3	3	2	3
Number of *mbs*	1	0	1	1
Number of *pds* on abominal segments I–VII	6	7	not presented	5
Pupa				
Number of setae on mesonotum	6	4	unknown	4
Number of *fes*	3	2	unknown	not presented
Number of setae on dorsum of abdominal segments I–VII	9	7	unknown	5–7

* This larva was probably misidentified by SCHERF (1964) and more likely belongs to the genus *Rhabdorrhnychus* (see the text for details).

Rhabdorrhnychus, which takes place in a root gall-like swelling (DIECKMANN 1983, STEJSKAL & TRNKA, unpubl. data).

MAY (1993) considered the increased number of *pds* on meso- and metathorax and abdominal segments I–VII and the increased number of epipharyngeal lining setae (*als*) (i.e. higher than the most frequent number of setae in weevils [for details see Chaetotaxy of mature larva of *C. nigrosuturatus*]) as diagnostic for the mature larva of the subfamily Lixinae. Descriptions of mature larvae from the tribe Lixini (*Larinus* species: GOSIK & SKUHROVEC 2011; *Lixus* species: SCHERF 1964, MAY 1994, NIKULINA 2001, 2007, NIKULINA & GÜLTEKIN 2011, GOSIK & WANAT 2014; *Rhinocyllus conicus*: MAY 1994) fit this diagnosis, as do all known species from the tribe Cleonini (see Table 1). The comparison of both tribes is recently not possible because our knowledge of immature stages in Cleonini is very scarce.

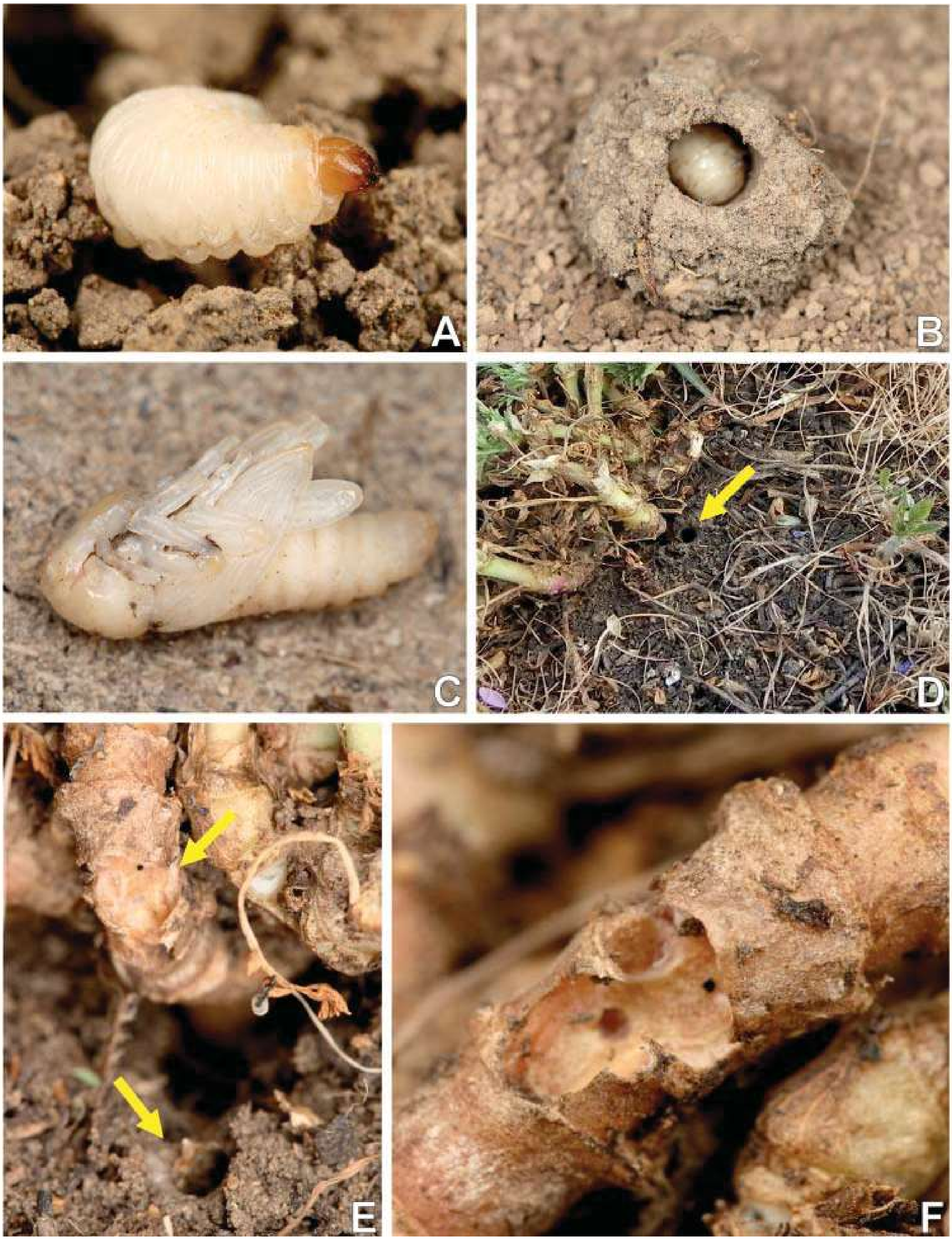

Fig. 4. Immature stages and feeding marks of *Coniocleonus nigrosuturatus* (Goeze, 1777). A – mature larva; B – earthen cell with mature larva; C – pupa (removed from the earthen cell); D – opening of larval tunnel in the soil; E – opening of the larval tunnel and feeding marks; F – detail of larval feeding marks.

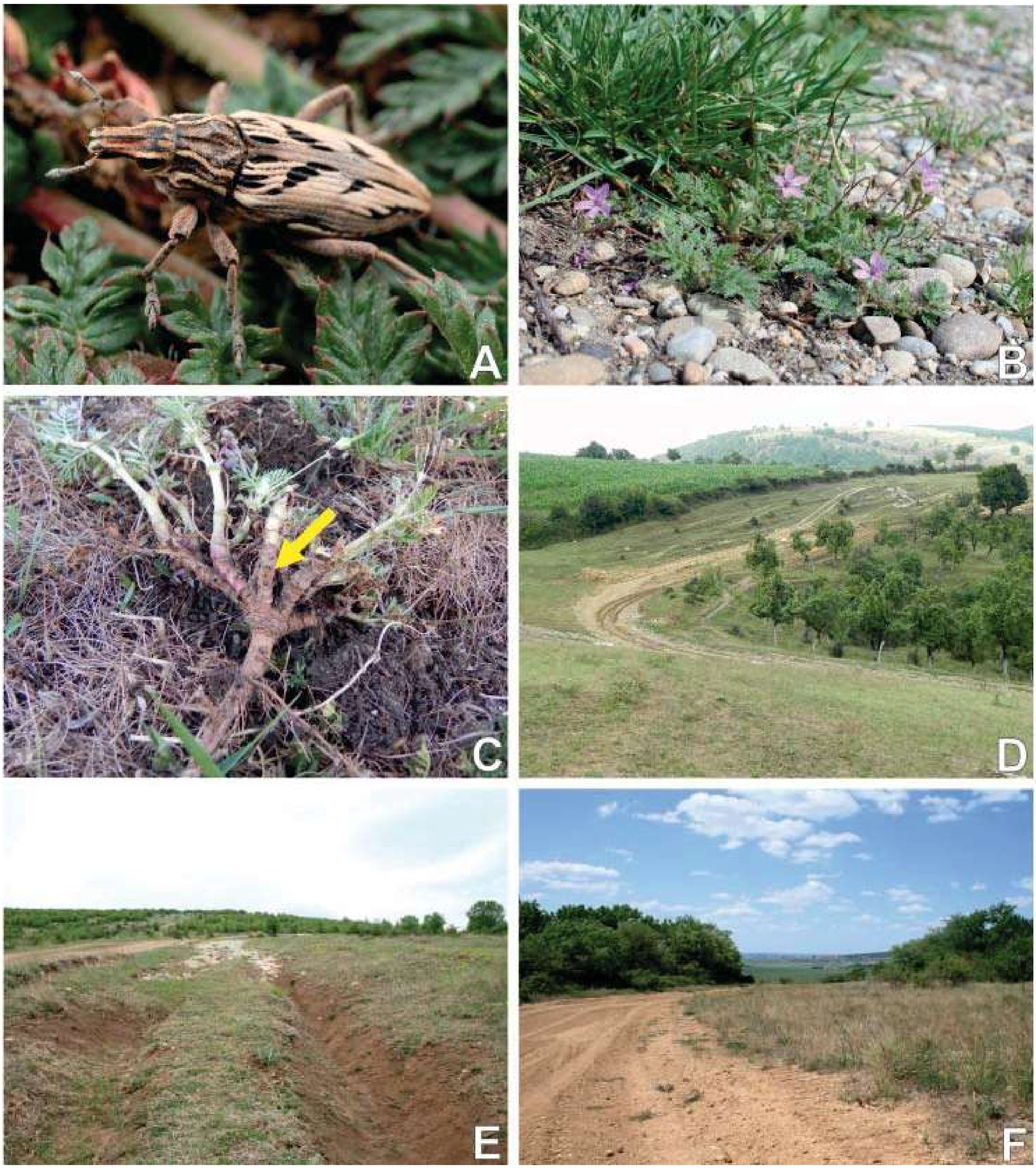

Fig. 5. Adult, host plant and habitats of *Coniocleonus nigrosuturatus* (Goeze, 1777). A – adult beetle; B – host plant *Erodium cicutarium* (L.) L'Hér.; C – an excavated host plant with larval feeding marks; D–E: habitats in Romania (Sfânta Elena); F – habitat in Slovakia (Kolíňany).

Biology of *Coniocleonus nigrosuturatus*

Habitat. Based on our observations, *C. nigrosuturatus* prefers dry, sunny places in grassland habitats with sparse vegetation, bare ground and patchily growing host plants. The weevil often inhabits road margins, pastures and dykes along rivers and requires a special habitat structure originating from a proper disturbation regime. Heterogeneous patches of properly disturbed sites occur for instance due to vehicle traffic (Figs 5E–F) or the grazing of domestic animals (Fig. 5D). The adults avoid places with compact and overgrown vegetation, where we suppose the soil is not sufficiently warm for larval development. Regarding bedrock, the weevil is quite flexible, occurring on a wide range of substrata, such as limestone and various sediments (sand, sandy gravel and loess).

Adult behaviour. The weevils are diurnal and can be very agile during sunny weather, however, during cold weather, they remain motionless. We usually observed adults (Fig. 5A) on the ground or on host plants, where they spend most of their time sitting on the top or underside of the host plant rosette (Fig. 5B). An attacked plant was usually occupied by 1–4 beetles. The adults were not observed in flight but are fully winged (Angelov 1963). Specimens examined by us for this character (in total 3 specimens from Hungary, Romania and Slovakia) were all macropterous. Adults can be observed from March onwards, with peak activity from mid-April to mid-May. Single adults can be observed at the end of summer or during autumn (August/October). We also observed beetles mating on the host plants. A mating male tightly embraces the female and repeatedly stimulates her with his mid-legs by knocking very rapidly on her metathorax.

Host plant. Both adults and larvae were observed feeding exclusively on Common Stork's-bill (*Erodium cicutarium* (L.) L'Hér.) (Geraniaceae, Figs 5B–C). Adults feed on leaves, whereas larval development occurs underground on the stem base. We also tested feeding on thyme (*Thymus* sp.), which was mentioned as a host plant by several authors (Kleine 1910, Fleischer 1927–1930, Fremuth 1982, Ter- Minasian 1988, Arzanov 2006). The adults completely avoided feeding on thyme, both in the laboratory and in the field, and we thus consider thyme solely as an occasional refuge plant. Other plant species were not tested. Based on our observations and experiments, *Coniocleonus nigrosuturatus* hence appears to be most likely monophagous, feeding exclusively on *Erodium cicutarium*.

Life cycle. *Coniocleonus nigrosuturatus* is a univoltine species. We did not find the site of egg deposition, but we suppose that females lay eggs in the soil near the root neck. Mature larvae live ectophagously near the host plant root and construct a vertical tunnel in the soil up to several centimeters long. The larval tunnel, which has a circular to oval shape in cross section with diameter of 4–5 mm, opens onto the soil surface, where the larvae feed externally on the stem base (Figs. 14D–F). We have not observed younger instar larvae, but we suppose that they most likely also live ectophagously, like the mature larvae. Their development inside roots/stems could be excluded because we did not find any elongated tunnels inside the plants despite exhaustive searching. No feeding marks were found on roots. At an attacked plant, a single larva was usually observed; however, sometimes up to 3 larvae were found. The host plant infestation rate may reach 50–75% according to our estimation. We observed higher larval feeding activity during the morning hours (before 10 a.m.), in the evening (after 7 p.m.) and in cloudy weather. During midday under warm and sunny conditions, larvae hid deeper in the tunnels. From the beginning of July, larvae pupate in oval, closed earthen cells, 25–30 mm long and 10–15 mm wide (Fig. 4B), located several centimeters below the soil surface under the host plant. The inner diameter of the earthen cell is approximately 5 mm, and its inner length is approximately 20 mm. The only reared adult emerged in the laboratory at the end of July. Further details of the life cycle were not observed. However, we suppose that a part of the population overwinters in the pupation cells, and some adults leave the cells at the end of summer, resulting in the records of adults in August to October (Dieckmann 1983, Stejskal & Trnka, unpubl.). Most likely, the weevils then spend some time feeding on the host plants and look for suitable shelters to overwinter in later.

Rearing of the larvae. In general, rearing of the larvae proved very difficult. We observed high larval mortality particularly due to the drying or decay of host plants. Only three larvae (of 25) pupated in the laboratory, of which one dried out, and another one was destroyed accidentally while examined. A fresh, not fully coloured adult was found in the earthen cell on July 22nd. After emergence, it remained in its pupation cell for at least a few days. The reared beetle was kept alive for observation but died on August 13th. The rearing of larvae on specially prepared host plants (technique 2 as described in Material and methods) was more succesfull because it prevented predation of the weevil larvae by carabid larvae taken accidentally from the field with the host plants.

Discussion

Erodium cicutarium, i.e. the exclusive host plant of *Coniocleonus nigrosuturatus* recorded in our study, is a very variable herbaceous annual or biennial plant, often regarded as a complex of taxa (Slavík 1997). This plant is supposed to be native to the Mediterranean, partially to western and northern Europe, and reaching central Asia (Slavík 1997). In central Europe, *E. cicutarium* is an archeophyte species. Recently, it has an almost worldwide distribution (Slavík 1997). It is frequently an important weed of cereal and other crops, particularly in semi-arid ranges (Francis et al. 2012). Using the weevil *Coniocleonus nigrosuturatus* as a potential biological control agent would be less effective, in our opinion, compared to some other Cleonini, such as *Cyphocleonus achates* (Fåhraeus, 1842) which feeds on invasive spotted knapweed (*Centaurea maculosa* Lam.) and diffuse knapweed (*Centaurea diffusa* Lam.) (Stinson et al. 1994). The larvae of *Cyphocleonus achates* mine the roots, causing extensive root damage, reduced shoot biomass or even death of attacked plants (Steinger & Müller-Schärer 1992, Corn et al. 2006). However, the larvae of *C. nigrosuturatus* live ectophagously on the underparts of stems and cause only negligible damage to the host plants, which appear perfectly adapted to disturbances, such as grazing, trampling or the feeding of larvae/adults. *Erodium cicutarium* also hosts other weevils in Europe, such as the root borer *Lixus vilis* (Rossi, 1790), the leaf-eating *Brachypera dauci* (Olivier, 1807), and *Limobius borealis* (Paykull, 1792) whose larvae feed on flower buds (Koch 1992). In studied localities, we often found *Coniocleonus nigrosuturatus*, *Lixus vilis* and *Brachypera dauci* sharing the same habitat and plant.

External larval feeding of *Coniocleonus nigrosuturatus* on the base of the stem is quite unique within Cleonini. A similar feeding place is known only for the larvae of *C. excoriatus* that feed at the junction of the stem and the upper taproot of *Emex spinosa* (Polygonaceae) (Yeoh et al. 2012). Cleonine larvae more typically feed on the exterior of roots (known for the genera *Asproparthenis*, *Pachycerus* and *Mecaspis*) or are endophagous inside the root/root neck (reported for *Cleonis*, *Cyphocleonus*, *Pseudocleonus* and *Rhabdorrhynchus*) (Scherf 1964; Koch 1992; Stejskal & Trnka, unpubl. data).

C. nigrosuturatus appears to be a characteristic species of grazed or even overgrazed habitats (Fadda et al. 2008). For example, this weevil was one of the dominant species of beetle communities in the plain of La Crau (southern France), which has had steppe-like vegetation managed by extensive sheep grazing since the Neolithic period with a high coverage of stones (Fadda et al. 2007, 2008). The weevil occurred only in dry grassland of the original steppe and was absent on formerly cultivated fields, which were used for the cultivation of melons and cereals until the 1970s. The abundance of *C. nigrosuturatus* was positively correlated with very short vegetation (< 5 cm), bare ground percentage cover and plant species richness. This is consistent with our data from Romania, where *C. nigrosuturatus* prefered heavily grazed and trampled places. However, *C. nigrosuturatus* is very sensitive to grazing abandonment, as reported by Fadda et al. (2008), based on long-term experiments on the effects of grazing abandonment on beetle communities in southern France. The authors documented that the abundance of *C. nigrosuturatus* was drastically decreased after only four years of grazing abandonment, and was very low after 23 years of abandonment. Although it is not mentioned in the paper, in our opinion, this may be connected with a change in the abundance of *Erodium*, which also tends to decrease after grazing abandonment (Fadda et al. 2008). We guess that grazing abandonment in warm areas with xerothermophilous steppe grasslands is the main cause of the sharp decline of *C. nigrosuturatus* in the Czech Republic, where this weevil is considered a critically endangered species (Benedikt & Strejček 2005, Stejskal & Trnka 2013). Nevertheless, our findings in Slovakia (a grassy parking place on a river bank) and Hungary (a closely cut lawn around a petrol station) demonstrate that this weevil can also survive, at least for some time, in ungrazed habitats, both semi-natural and secondary ones. We guess that a proper regime of disturbances that retain sufficient bare ground in the habitat is essential for both *C. nigrosuturatus* and its host plant.

Knowledge of immature stages and life histories can help more effectively protect endangered species (including the species presented here). The detailed description of the larva and pupa and its comparison with known descriptions reported here, demonstrates the possibility of identifying immatures in these species, as has been done in other groups (Hyperini: Skuhrovec 2006, 2007; *Bagous*: Gosik 2008; Ceutorhynchinae: Gosik 2010; Lixinae: Nikulina 2001, 2007, Nikulina & Gültekin 2011, Gosik & Skuhrovec 2011, Gosik & Wanat 2014; Entiminae: Gosik & Sprick 2012a,b, 2013, Tychiini: Skuhrovec et al. in press). In practice, species identification of larvae with chaetotaxy is relatively easy, and is generally much less expensive than identification using molecular methods (Hirsch et al. 2010). Unfortunately, the relatively low number of available descriptions in comparison to the great number of known adult weevil species represents a current problem in the taxonomic use of the immature stages.

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PŘÍLOHA III

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Biology and morphology of immature stages of *Adosomus roridus* (Coleoptera: Curculionidae: Lixinae)

FILIP TRNKA[1], ROBERT STEJSKAL[2] & JIŘÍ SKUHROVEC[3]

[1] *Department of Ecology & Environmental Sciences, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic. E-mail: filip.trnka88@gmail.com*

[2] *Administration of Podyji National Park, Na Vyhlídce 5, CZ-669 02 Znojmo, Czech Republic. E-mail: rstejskal@centrum.cz*

[3] *Group Function of Invertebrate and Plant Biodiversity in Agrosystems, Crop Research Institute, Drnovská 507, CZ-161 06 Praha 6–Ruzyně, Czech Republic. E-mail: jirislavskuhrovec@gmail.com*

Abstract

Mature larva and pupa of *Adosomus* (s. str.) *roridus* (Pallas, 1781) (Curculionidae: Lixinae: Cleonini) are described and compared with ten other taxa of Cleonini with known larvae. This weevil is an oligophagous species on the Asteraceae family. From our observations in Slovakia, we found active adults from April to September in dry sunny places within extensively used or fallow vineyards and in ruderal vegetation with host plants. The weevil is a root borer - larvae, pupae and fresh adults were collected from the root necks and roots of Common Tansy (*Tanacetum vulgare* L.) and rarely from Mugwort (*Artemisia vulgaris* L.). Each plant was usually occupied by one larva, or more rarely with two or three larvae. The new generation of adult individuals appeared from early summer to autumn. Both larvae and some of the adults overwinter, which is quite unique within Cleonini.

Key words: mature larva, pupa, host plant, Asteraceae, *Artemisia*, *Tanacetum,* life history, central Europe, Palaearctic region

Introduction

Weevils from the subfamily Lixinae Schoenherr, 1823 (Coleoptera, Curculionidae) are medium or large-sized beetles mainly found in the Palaearctic region, especially in Asia. The subfamily contains approximately 1,500 species (Meregalli 2014). Lixinae is sometimes regarded as part of Molytinae (Oberprieler *et al.* 2007) or Curculioninae (Lawrence & Newton 1995), but more often, Lixinae is considered a separate subfamily (e.g., Alonso-Zarazaga & Lyal 1999, Löbl & Smetana 2013). Lixinae belong to fairly derived weevil groups, and in general, they are separated into two tribes: Cleonini Schoennherr, 1826 and Lixini Schoenherr, 1823 (Löbl & Smetana 2013); Meregalli (2014) divides the groups into three tribes, with the third tribe being Rhinocyllini Lacordaire, 1863. Although general phylogenetic analysis of the entire tribe is still in progress (Meregalli 2014, Meregalli & Silvestro unpubl.), there are some preliminary results for Mediterranean and Afrotropical taxa (Meregalli & Silvestro 2008). Until recently, the immature stages of Lixinae were largely unknown and understudied (Anderson 1987). Current knowledge mainly focuses on the Lixini, for which several studies describing the morphology of immature stages have been published (*Larinus* Dejean species: Zotov 2009a, 2010; Gosik & Skuhrovec 2011; *Lixus* F. species: Scherf 1964; Lee & Morimoto 1988; Nikulina 2001, 2007; Zotov 2009a, b; Nikulina & Gültekin 2011; Gosik & Wanat 2014; Skuhrovec & Volovnik in press; *Rhinocyllus conicus* (Frölich, 1792): May 1994). Knowledge of the immature stages of Cleonini remains limited because the immature stages of only 10 species have been described in detail, by Hoffmann (1950), Scherf (1964), Zotov (2011) and Stejskal *et al.* (2014).

The Palaearctic genus *Adosomus* Faust, 1904 belongs to the Cleonini tribe and comprises 9 known species (Meregalli & Fremuth 2013). This genus is classified into three subgenera: *Adosomus* Faust, 1904 (2 species), *Pseudoadosomus* Arzanov, 2005 (4 sp.) and *Xeradosomus* Arzanov, 2005 (3 sp.). The only European species is *A.*

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roridus (Pallas, 1781), belonging to the subgenus *Adosomus* and distributed from south Sweden through central Europe, Slovenia, and Italy to Eastern Europe and Kazakhstan. Other species can be found in central Asia, Siberia, Mongolia and China (Meregalli & Fremuth 2013).

Data regarding host plants of *Adosomus* species are limited. For some species, the only information about the refuge plants is their hiding or feeding location. Larvae of an *Adosomus* species from China were found in the stems and roots of *Artemisia ordosica* Krasch. (as *A. ordosoca*) and *A. sphaerocephala* Krasch. (Wang *et al.* 2009). *Adosomus roridus* is a very rare and endangered species in central Europe, and there is very little information on its bionomy (Stejskal & Trnka 2013). This species prefers xerothermic steppes and disturbed sites with *Artemisia* L. and *Tanacetum* L. (Würmli 1976, Koch 1992, Mazur 2002). Immature stages of an unidentified *Adosomus* species from China are known (Wang *et al.* 2009) but are not described. The immature stages of *A. roridus* have never been observed. In this paper, we describe the immature stages of the species and provide details on their life history based on observations made during field work in Slovakia.

Materials and methods

The material used to describe immature stages was collected, and field observations were conducted, in the following locality:

Material examined. SLOVAKIA: Bratislava region: Pezinok-Grinava env., 48°16'9.66"N, 17°13'52.95"E. Visit dates: 6.xi.2012, 15.v.2014. Bratislava region: Svätý Jur env., 48°14'36.72"N, 17°11'55.34"E. Visit dates: 15.v.2014, 13.vii.2014. Habitats: Vineyards and road margins. Bedrock: quaternary alluvial sediments (sandy gravel). Altitude: 175 m a.s.l. (see Fig. 5C–E).

Rearing and life cycle observations were conducted during the 2012-2013 and 2014-2015 vegetation seasons. Laboratory observations were conducted in Olomouc, Czech Republic (49°35'36"N, 17°15'3"E). Field work consisted of larva collection (performed by F. Trnka) and the study of host plant associations. Root necks or roots inhabited by one or more larvae were collected and then cultivated in a common flowerpot. The plants were placed into a sandy substrate and moisturised once a week.

Part of the larval and pupal material was preserved in Pampel fixation liquid (4 parts glacial acetic acid, 6 parts 4% formaldehyde, 15 parts 95% ethyl alcohol and 30 parts distilled water) and used for the morphological descriptions. These specimens are now deposited in the Group Function of Invertebrate and Plant Biodiversity in Agrosystems of the Crop Research Institute (Prague, Czech Republic). Plants were identified by the collectors.

To prepare slides (for details, see May (1994)), a larva was decapitated, and its head was rinsed in distilled water and cleared in a 10% potassium hydroxide (KOH) solution. After clearing, the mouth parts were separated from the head capsule. The head capsule and all mouth parts were mounted on permanent microscope slides in Euparal. All other body parts were mounted on temporary microscope slides in 10% glycerine.

The observations and measurements were made using a light microscope with calibrated oculars (Olympus BX 40 and Nikon Eclipse 80i). The following characteristics were measured for each larva (head width, length of the body (larvae fixed in a C-shape were measured in segments), width of the body in the widest place (metathorax or abdominal segments I-IV)), and pupa (length and width at the widest place). The thorax and abdomen are not sclerotised, and it was unlikely that the fixation process altered the weevils' proportions; measurements of these parts are given for comparison purposes only.

Drawings were made with a drawing tube on a light microscope and processed by a computer (Adobe Photoshop, Corel Photo-Paint 11, GIMP 2). The thoracic spiracle is placed on the prothorax near the boundary of the prothorax and mesothorax, as shown in the drawing (see Fig. 2A), but it is of mesothoracic origin (Marvaldi *et al.* 2002; Marvaldi 2003). The drawings show the thoracic and abdominal spiracles (see Figs. 2A-C). The numbers of setae of the bilateral structures are given for one side.

We used the terms and abbreviations for the setae of the mature larva and pupa studied in Scherf (1964), May (1977, 1994) and Marvaldi (1998, 1999).

The naming (and hence count) of some setae on the epipharynx (especially *ams* and *mes*) is not completely in agreement among authors. According to May (1994: 376) and Marvaldi (1998, 1999) the standard state for the epipharynx in weevils is 2 *ams* and 3 *mes*, but the position of the distal pair of *mes* is often very close or in line with the *ams* and its apical shape identical with *ams*. Consequently some authors interpret and count the former as being *ams*. The decision was finally made to add this problematic seta to the latter group (*ams*). The position of this seta is still not resolved in our view, and despite it being almost absolutely identical as in *Coniocleonus* Motschulsky or *Tychius* Germar, we have not followed Stejskal *et al.* (2014) and Skuhrovec *et al.* (2014), who accepted the standard status in weevils and counts it as a *mes* seta.

Morphology of mature larva and pupa

Adosomus (s.str.) *roridus* (Pallas, 1781)

Material examined. SLOVAKIA: Bratislava region: Pezinok-Grinava env., (48°16'9.66"N, 17°13'52.95"E). 27.v.2012 (1 pupa (♂)), 15.v.2014 (4 larvae, 1 pupa (♀)), 13.vii.2014 (1 pupa (♂)), all leg. F. Trnka.

Description of mature larva. COLOURATION. Brown or dark brown head with a distinct pale pattern around the frontal suture and lateral sides (Fig. 4C). All thoracic and abdominal segments white; only dorsum of pronotum with elongated light brown stripe (Figs. 4C–D).

HEAD CAPSULE AND MOUTH PARTS. Head width: 2.5–3.0 mm (mean 2.8 mm), suboval, flattened laterally, endocarinal line long exceeded almost to the half of frons. Frontal sutures on head distinct, extended to antennae. Single stemma (st), in the form of a dark pigmented spot, located on each side anterolaterally. *Des1* and *des2* located in upper part of the central part of epicranium, *des1* near to the middle part of epicranium, and *des2* near to side of epicranium, *des3* located anterially near to frontal suture, *des4* located in the central part of epicranium, *des5* located anterolaterally; all *des* long, almost all equal in length (Fig. 1A). *Fs1* and *fs2* placed medially, *fs3* located anteriomedially, *fs4* located anteriolaterally, and *fs5* located laterally, close to the epistoma; all setae relatively long, *fs5* slightly longer than the remaining four setae (Fig. 1A). *Les1–2* as long as *des1*; and *ves1–3* short. Epicranial area without pores. Antennae located at the end of the frontal suture on each side, membranous and slightly convex basal article bearing conical triangular sensorium, relatively long; basal membranous article with 3 sensillae different in both shape and length (Fig. 1D).

Clypeus (Fig. 1E) approx. 2.2 times as wide as long with 2 relatively long *cls*, almost equal in length, localized posteriolaterally and 1 sensillum; anterior margin rounded to the inside; median part covered by thorn-shaped cuticular processes. Labrum (Fig. 1E) approximately 4 times as wide as long, with 3 pairs of hairform *lms*, of different length; *lms3* distinctly shorter than very long *lms1* and *lms2*; *lms1* placed close to the margin with clypeus, *lms2* located anteriomedially and *lms3* located anteriolaterally; anterior margin double sinuate. Epipharynx (Fig. 1F) with 4 blunt, finger-like *als*, unequal in length, 2 laterally *als* distinctly shorter than 2 medially *als*; 3 very short. blunt, finger-like *ams*, *ams1* and *ams2* distinctly larger than *ams3*; 2 pairs of short, blunt *mes*, unequal in length; labral rods (lr) rather broad, sub-triangular, approximating towards base. Mandibles (Fig. 1C) distinctly broad, bifid, tooth of unequal height; slightly truncate; both *mds* relatively long, hairform, located in distinct holes. Maxilla (Fig. 1B) stipes with 1 *stps*, 2 *pfs* and 1 *mbs*, *stps* and *pfs1–2* very long, equal in length, *mbs* very short; mala with 12 bacilliform *dms* in two different lengths (6 very long and 6 relatively long); 5 short *vms*, almost equal in length; *vms* distinctly shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with 1 very short *mxps* and two sensilla; length ratio of basal and distal palpomeres: 1:0.9; distal palpomere with one sensillum and a group of conical, cuticular apical processes. Praelabium (Fig. 1B) heart-shaped and distinctly elongated, with 1 very long *prms*; ligula with sinuate margin and 3 hairform short *ligs*, unequal in length; premental sclerite well visible. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres: 1:0.8; distal palpomere with one sensillum and short, cuticular apical processes; basal palpomere with 1 dorsal sensillum. Postlabium (Fig. 1B) with 3 *pms*, *pms1* located anterially, remaining two pairs laterally; *pms1* and *pms2* very long, *pms3* distinctly shorter; surface of postlabium partly covered by distinct cuticular processes.

THORAX AND ABDOMEN. Body length: 12.0–16.4 mm (mean 14.5 mm) stocky, slightly curved, rounded in cross section (Fig. 2A). The widest place in the body (metathorax and abdominal segments I–IV) measuring up to 6.0 mm. Prothorax distinctly smaller than meso- and metathorax. Metathorax and abdominal segments I–IV of almost equal length, next abdominal segments decreasing gradually to the terminal parts of the body. Abdominal segment X reduced to four anal lobes of unequal size, the dorsal being distinctly the largest, the lateral pair equal in size, and the ventral lobe very small. Anus located terminally. Spiracles (9 pairs) unicameral, the first placed between the pro- and mesothorax (see Material and methods), the abdominal spiracles located laterally, close to the anterior margin of abdominal segments I–VIII.

Chaetotaxy of mature larva. Setae thin, short to relatively long, light yellow or orange. **Thorax.** Prothorax (Fig. 2B) with 11 *prns* unequal in length, 8 of them on weakly pigmented dorsal sclerite, this sclerite subdivided in two triangular plates medially, 3 of them closely to spiracle; 2 relatively long *ps* and 1 *eus*. Mesothorax (Fig. 2B) with 1 *prs*; 5 *pds* unequal in length, *pds1–3* and *pds5* long, *pds4* very short; 1 relatively long *as*; 3 *ss* unequal in length, *ss1* and *ss2* short, *ss3* very short; 1 relatively long *eps*; 1 relatively long *ps* and 1 short *eus*. Chaetotaxy of metathorax (Fig. 2B) almost identical to mesothoracal, metathorax with only 2 *ss* almost equal in length, both short. Each pedal area of thoracic segments well separated, with 6 relatively long *pda*, 3 of them on pigmented pedal area, unequal in length. **Abdomen.** Abdominal segments I–VII (Figs 2C–D) with 1 short *prs*; 6 relatively long *pds*, *pds5–6* the longest one, *pds3* the shortest; 2 *ss* of unequal length, *ss1* very short, *ss2* long as *pds6*; 2 relatively long *eps* of almost equal length; 2 relatively long *ps* of equal length; 1 short *lsts* and 2 short *eus*. Abdominal segment VIII (Fig. 2D) with 1 short *prs*; 5 *pds, pds6* lacking, *pds3* less than half of length of the four relatively long remaining setae; 2 *ss* of unequal length, *ss1* very short, *ss2* long as *pds5*; 2 relatively long *eps* of almost equal length, 2 relatively long *ps* of equal length, 1 short *lsts* and 2 short *eus*. Abdominal segment IX (Fig. 2D) with 5 *ds* (*ds1,3,5* long, *ds2,4* short); 3 *ps* of unequal length, *ps1* very short, *ps2–3* almost long as *ds1* and 2 very short *sts*. Ventral anal lobe on abdominal segment X (Fig. 2D) with 2-3 short seta (*ts*).

Description of pupa. COLOURATION. Body whitish to yellowish.

MORPHOLOGY (Figs. 3A–C, 4F). Body length: 13.0–17.5 mm (♂ 13.5–17.5 mm; ♀ 13.0 mm), at the widest region: 4.8–6.0 mm. The widest place in the body is commonly between the apex of the meso- or metafemora. Body stocky. Cuticle smooth. Rostrum short and wide, approximately 1.5 times as long as wide, extended to procoxae. Antennae relatively long and stout. Pronotum from 1.3 to 1.4 times as wide as long. Mesonotum and metanotum of almost equal length. Abdominal segments I–IV of almost equal length; abdominal segment V semicircular, next abdominal segments diminish gradually to the end of the body. Abdominal segments VI–IX distinctly smaller than other abdominal segments. Gonotheca (abdominal segment IX) in females (1 specimen) divided. Sexual dimorphism in weevils is visible mainly in the length of rostrum and in the structure of abdominal segment IX: gonotheca of ♂ undivided, of ♀ divided.

CHAETOTAXY (Figs. 3A–C). Setae short to very short, unequal in length, light yellow or orange, some setae on abdominal segments I–VIII distinctly get stronger and located on protuberances. Setae well visible. Head capsule includes only 1 *vs*, 3 *sos* and 4 *pas*. Rostrum with 2 *rs*. Setae on head capsule straight, as short as the remaining setae on thoracic and abdominal segments. Pronotum with 2 *as*, 2 *ds*, 2 *ls* and 4 *pls*, *ds1* distinctly shorter than the remaining setae. Dorsal parts of mesothorax with 1 seta located posteromedially, 1 seta posterolaterally and 4(5) setae located along its anterior margin. Dorsal parts of metathorax with 4 setae located along its anterior margin. Each apex of femora with groups of 2 *fes*. Dorsal parts of abdominal segments I–VIII each with 2 setae located posteriorly (*d1*, *d10*) and 8 pairs (*d2–9*) located along theirs anterior margins. Setae *d2–4* (on abdominal segment I)*, d2–6* and *d8* (on abdominal segment II), *d2–8* (on abdominal segment III–VII) short, thorn-like, located on protuberances. Remaining setae short, hair-like. Abdominal segments I–VII with groups of 2 lateral setae and 5 ventral setae (4 setae on abdominal segment VII). Dorsal part of abdominal segment VIII with 2 setae located posteriorlly (*d1*, *d10*) and 8 (*d2–9*) located along its anterior margin; *d2,* and *d3* thorn-like, located on protuberances; remaining setae short. Abdominal segment VIII with groups of 2 lateral setae and 4 short ventral setae. Abdominal segment IX with 2 ventral microsetae and 1 short, thin seta. Pseudocerci short, triangular, with 2 spines, and 3 ventral very short and 2 micro, thin setae.

Comparison with larvae of other Cleonini. The larvae of ten cleonine taxa have already been described (Hoffmann 1950, Scherf 1964, Zotov 2011, Stejskal *et al.* 2014). The detailed description of the pupa is similar for nine cleonine taxa (Scherf 1964, Zotov 2011, Stejskal *et al.* 2014). The comparison of the larva and pupa of *Adosomus roridus* with those described by Scherf (1964) was somewhat difficult due to the use of different terminology for morphology and chaetotaxy and/or the absence of good quality drawings (more in Stejskal *et al.* 2014). The comparison of the larva with *Leucomigus tesselatus* (Fairmaire, 1849) described/ drawn by Hoffmann (1950) is somewhat challenging due to the existence of only a few drawings, and some positions of setae are suspicious (e.g., number of *fs*, *cls* and *mbs*; see Skuhrovec *et al.* 2014, 2015). The descriptions of five cleonine larvae and pupae by Zotov (2011) are of higher quality and very useful; however, the described characters are only useful for differential diagnosis. The detailed descriptions of these five cleonine taxa are missing. Despite these challenges, we were able to compare the morphology of all ten taxa (Table 1).

Table 1. Differential diagnosis of mature larvae and pupae of 11 species from the tribe Cleonini.

	Adosomus roridus	***Asproparthenis carinicollis***	***Bothynoderes affinis***	***B. declivis***	***Coniocleonus nigrosuturatus***	***Cleonis pigra***	***Cyphocleonus achates***	***C. dealbatus***	***Leucomigus tesselatus***	***'Pachycerus scabrosus'* ***	***Rhabdorrhynchus karelinii***
Larva											
Endocarina	present	present	present	present	absent	present	present	absent	absent	absent	present
Number of *des*	5	5	4	4	5	4	5	5	5	5	5
Number of *fs*	5	5	5	5	5	4	5	5	2	5	5
Number of *les*	2	2	2	2	2	not presented	2	3	2	3	3
Number of *ves*	3	not presented	not presented	not presented	2	not presented	not presented	not presented	not presented	not presented	not presented
Number of *cls*	2	3	2	2	2	2	2	2	3	3	2
Position of *lrms1-3*	in a triangle	in a triangle	in a line	in a line	in a triangle	in a triangle	in a line	in a triangle	in a line	in a line	in a line
Number of *als*	4	3	3	3	4	4	3	6	3	3	3
Number of *mds*	2	1	1	1	1	2	1	2	2	2	2
Number of *ligs*	3	2	2	2	3	3	2	2	3	3	3
Number of *mbs*	1	1	1	0	1	0	1	1	2	1	0
Number of *pds* on Abd. seg I- VII	6	4	6	6	6	7	6	not presented	5	5	6
Pupa											
Number of setae on mesonotum	6(7)	3	3		6	4	3	Unknown	Unknown	4	6
Number of *fes*	2	2	2		3	2	2	Unknown	Unknown	not presented	2
Number of setae on dorsum of Abd. seg. I-VII	10	8	6		9	7	9	Unknown	Unknown	5-7	10

* This larva was probably misidentified by Scherf (1964) and more likely belongs to the genus *Rhabdorrhnychus* (see the text for details).

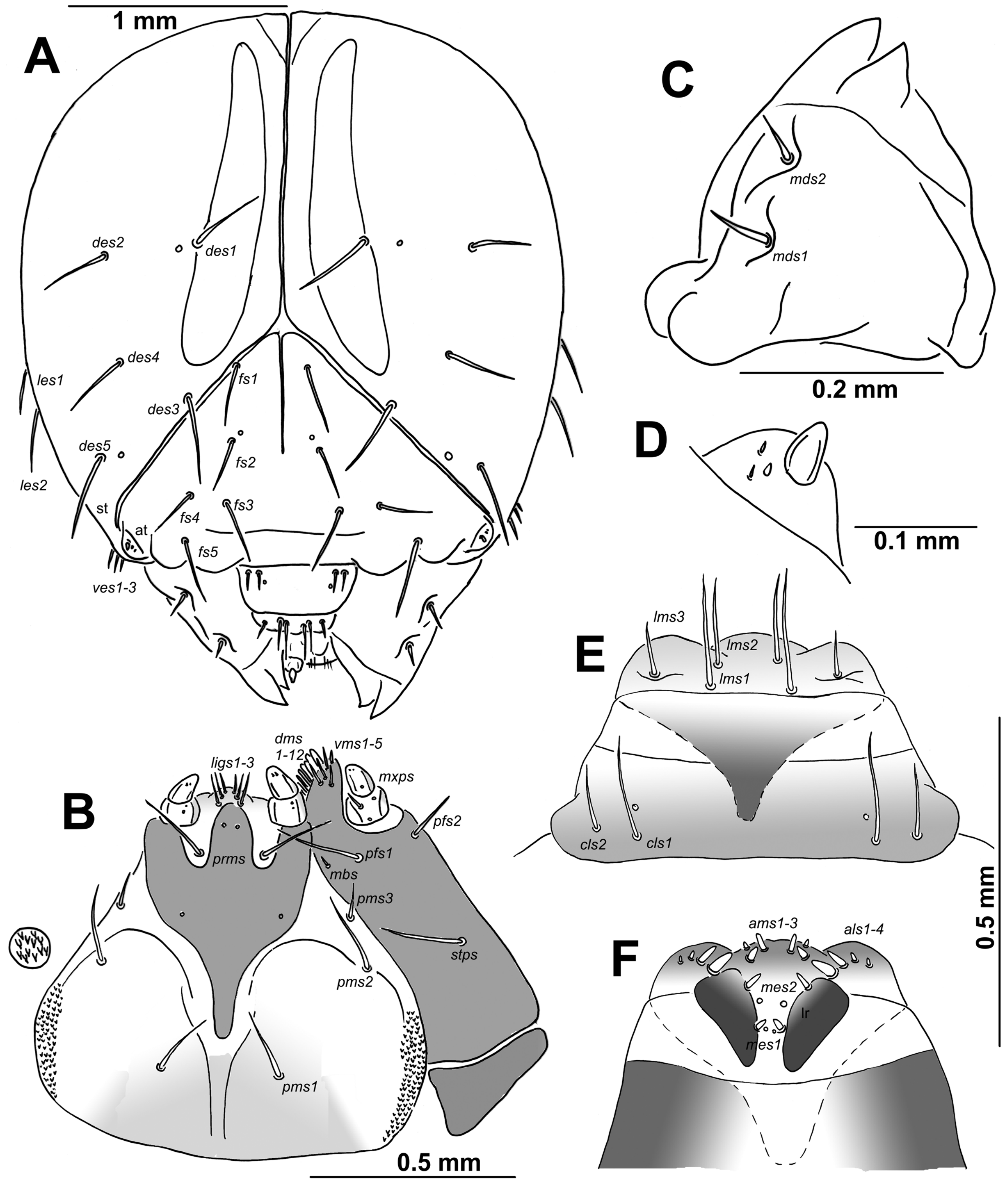

FIGURE 1. *Adosomus roridus* (Pallas, 1781), mature larva head, antenna and mouth parts: A—dorsal view (*des*—dorsal epicranial seta (e), *fs*—frontal epicranial s., *les*—lateral epicranial s., *ves*—ventral epicranial s., st—stemmata, at—antenna); B—right maxilla, dorsal view (*dms*—dorsal malar s., *vms*—ventral malar s., *mpxs*—maxillary palps s., *mbs*—basoventral s., *pfs*—palpiferal s., *stps*—stipital s.), prementum and postmentum, ventral view (*prms*—premental s., *pms*—postmental s., *ligs*—ligular s.); C—right mandible (*mds*—mandible dorsal s.); D—antenna; E—labrum and clypeus (*cls*—clypeal s., *lms*—labral s.); F—epipharynx (*ams*—anteromedial s., *als*—anterolateral s., *mes*—median s., lr—labral rods).

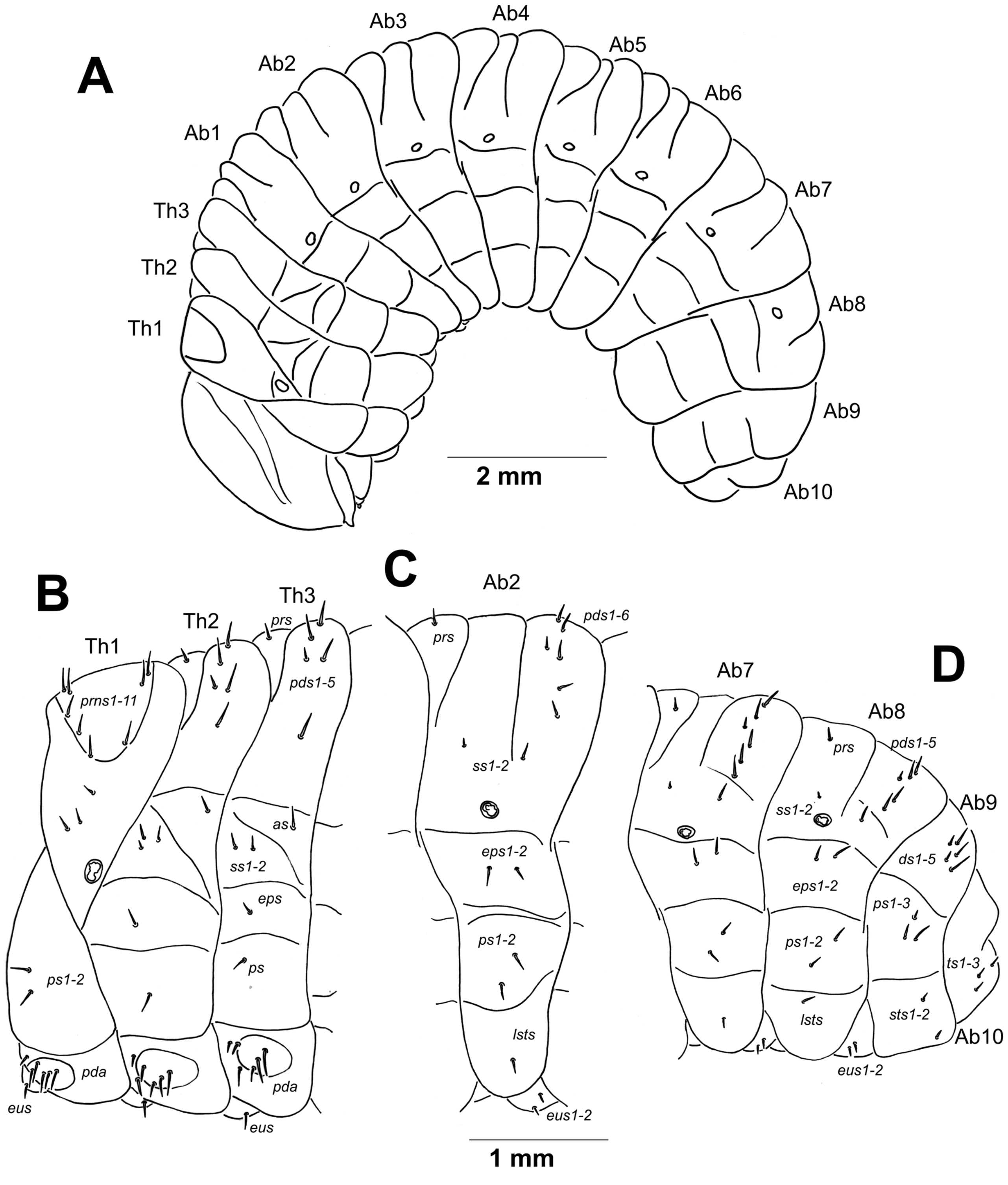

FIGURE 2. *Adosomus roridus* (Pallas, 1781), mature larva habitus: A—shape of the body (lateral view), B—lateral view of thoracic segments, C—lateral view of abdominal segment II, D—lateral view of abdominal segments VII–X (*prns*—pronotal seta(e), *prs*—prodorsal s., *pds*—postdorsal s., *as*—alar s., *ss*—spiracular s., *eps*—epipleural s., *ps*—pleural s., *pda*—pedal s., *lsts*—laterosternal s., *eus*—eusternal s., *ds*—dorsal s., *sts*—sternal s., *ts*—terminal s.; Th1-3, Ab1-10—number of thoracic and abdominal segment).

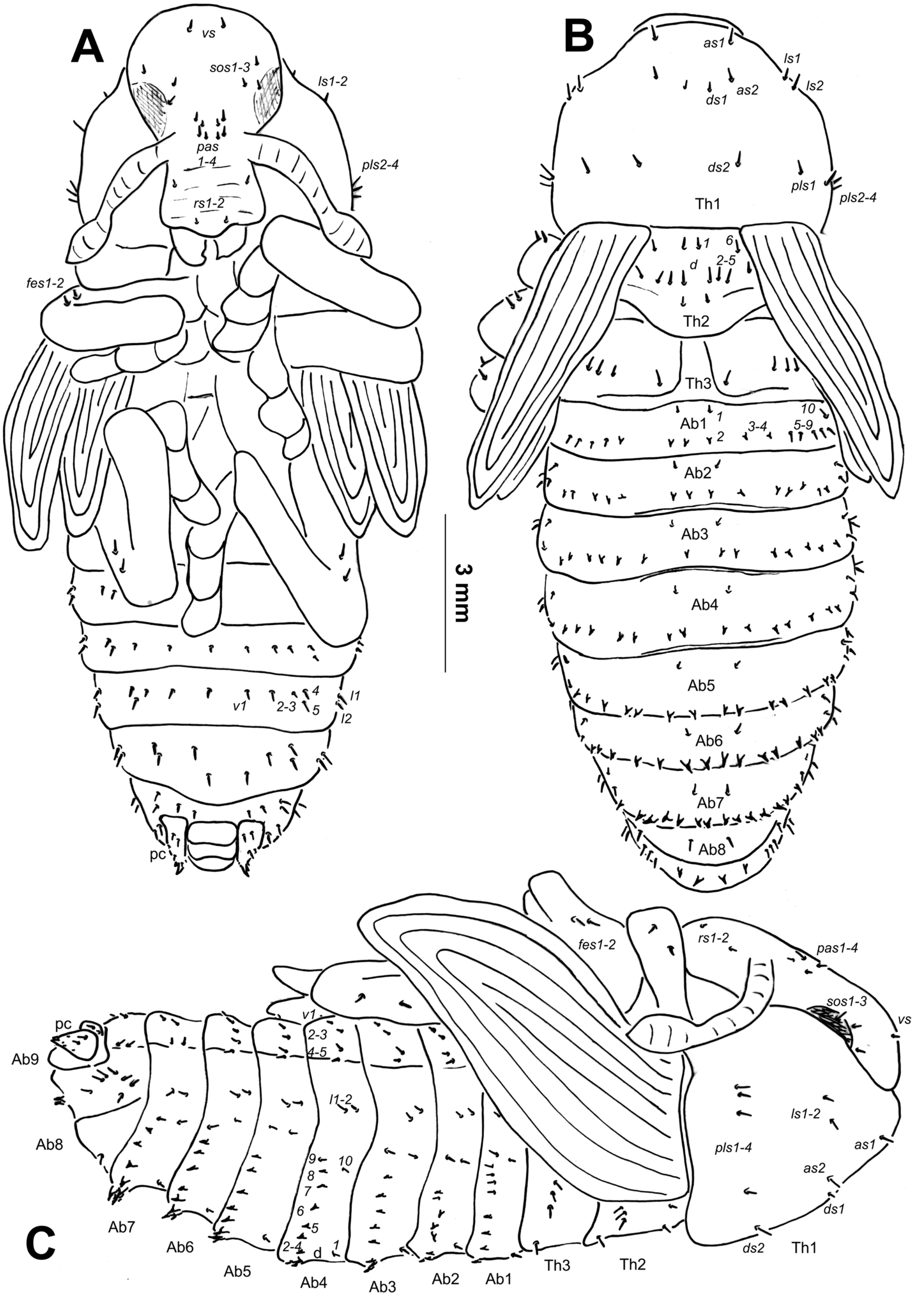

FIGURE 3. *Adosomus roridus* (Pallas, 1781), pupa habitus: A—ventral view, B—dorsal view, C—lateral view (*vs*—vertical seta (e), *sos*—superorbital s., *pas*—postantennal s., *rs*—rostral s., *as*—apical s., *ls*—lateral s., *ds*—discal s., *pls*—posterolateral s., *fes*—femoral s., *d*—dorsal s., *l*—lateral s., *v*—ventral s.; Th1-3, Ab1-9—number of thoracic and abdominal segment, pc—pseudocerci).

May (1993) considered the increased number of *pds* on meso- and metathorax and abdominal segments I–VII and the increased number of epipharyngeal lining setae (*als*) (i.e., higher than the most frequent number of setae in weevils) (for details, see Stejskal *et al.* 2014**)** as diagnostic for the mature larva of the subfamily Lixinae. Descriptions of mature larvae from the Lixini (*Larinus* species: Zotov 2009a, 2010; Gosik & Skuhrovec 2011; *Lixus* species: Scherf 1964; Lee & Morimoto 1988; Nikulina 2001, 2007; Zotov 2009a, b; Nikulina & Gültekin 2011; Gosik & Wanat 2014; Skuhrovec & Volovnik in press; *Rhinocyllus conicus*: May 1994) fit this diagnosis, as do all known species from the Cleonini tribe (Stejskal *et al.* 2014). Gosik & Wanat (2014) compared the larvae and pupae of genera *Lixus* and *Larinus* and determined their differential characters. The precise key, detailed generic study of the Cleonini tribe and the comparison of both tribes was not possible because of our limited knowledge of the immature stages.

Biology of *Adosomus roridus*

Habitat. Adults prefer dry, sunny places with host plants. In our study area, this species inhabited extensively used or fallow vineyards (Figs. 5D–E), ruderal vegetation and road edges in the vicinity (Fig. 5C). They inhabited both early and older succession stages of ruderal biotopes. Both adult individuals and immature stages were found in places with sparse vegetation, as well as in places with dense ruderal vegetation. Vineyard rows, where adults were often found, were manually or mechanically cultivated from time to time.

Adult behaviour. We usually observed adults under the host plant's rosette (Fig. 4A) near the stem base. The adults are diurnal, and during sunny weather, they may climb up the host plant, even reaching the flowers. At night and in cold weather, these beetles are hidden under the rosettes of host plants. This species has very reduced wings (five specimens examined) and thus is most likely flightless. Adults can be observed from April to September. Published records of adults are available from the beginning of April to September (Roubal 1937–1941, Kofler 1962–63). According to these findings, both larvae and some adults overwinter.

Host plants. Only adults of *Adosomus roridus* are known to feed on several Asteraceae: *Artemisia absinthium* L., *A. vulgaris* L. and *Tanacetum vulgare* L. (Smreczyński 1968, Dieckmann 1983, Koch 1992). We observed that adults, especially at *Tanacetum vulgare* (Fig. 5A) and less at *Artemisia vulgaris* (Fig. 5B), preferred larger and older polycormons.

Life cycle. A female weevil bites the root neck/root of the host plant and lays an egg in the hole. The egg is 3 mm long and yellow (Fig. 4B). A mature larva lives endophagously in the root neck or root where it feeds on plant tissues (Figs. 4C–D). During its development, the larva causes a swelling of the root (Fig. 4E). Usually, only one larva occupies a plant, but rarely, there were 2–3 larvae located on one plant; each larva created its own chamber. Mature larvae were found from the beginning of May to the second half of July. According to our observations, larvae of different instars overwinter and pupate the next year. Pupation occurs in the root neck/root. The new generation of adult beetles emerge from early summer to autumn. Some adults also overwinter, from early spring (beginning of April), which is supported by published data (Roubal 1937–1941, Kofler 1962–63).

Rearing of the larvae. For laboratory breeding, 15 larvae were collected. Only older instars were able to be reared. Younger instars were hard to rear because the host plant root had a tendency to dry out and reduce its size, which led to destruction of the larva.

Discussion

The weevil *Adosomus roridus* is an oligophagous species on Asteraceae. In our study, we found adults and immature stages on *Tanacetum vulgare* and *Artemisia vulgaris* with a preference of the former. Immature stages live endophagously, similar to most of the other Cleonini, which are typical root borers e.g., *Bothynoderes* Schoenherr, *Cleonis* Dejean, *Cyphocleonus* Motschulsky or *Leucomigus* Motschulsky (Volovnik 2010). Conversely, most members of Lixini are stem borers (e.g., *Lixus*) or develop in flowers, e.g., *Bangasternus* Gozis, *Larinus* or *Lachnaeus* Schoenherr (Stejskal & Trnka 2014). In some genera, however, there are exceptions, e.g., *Lixus angustus* (Herbst, 1795) as a root borer (Dieckmann 1983).

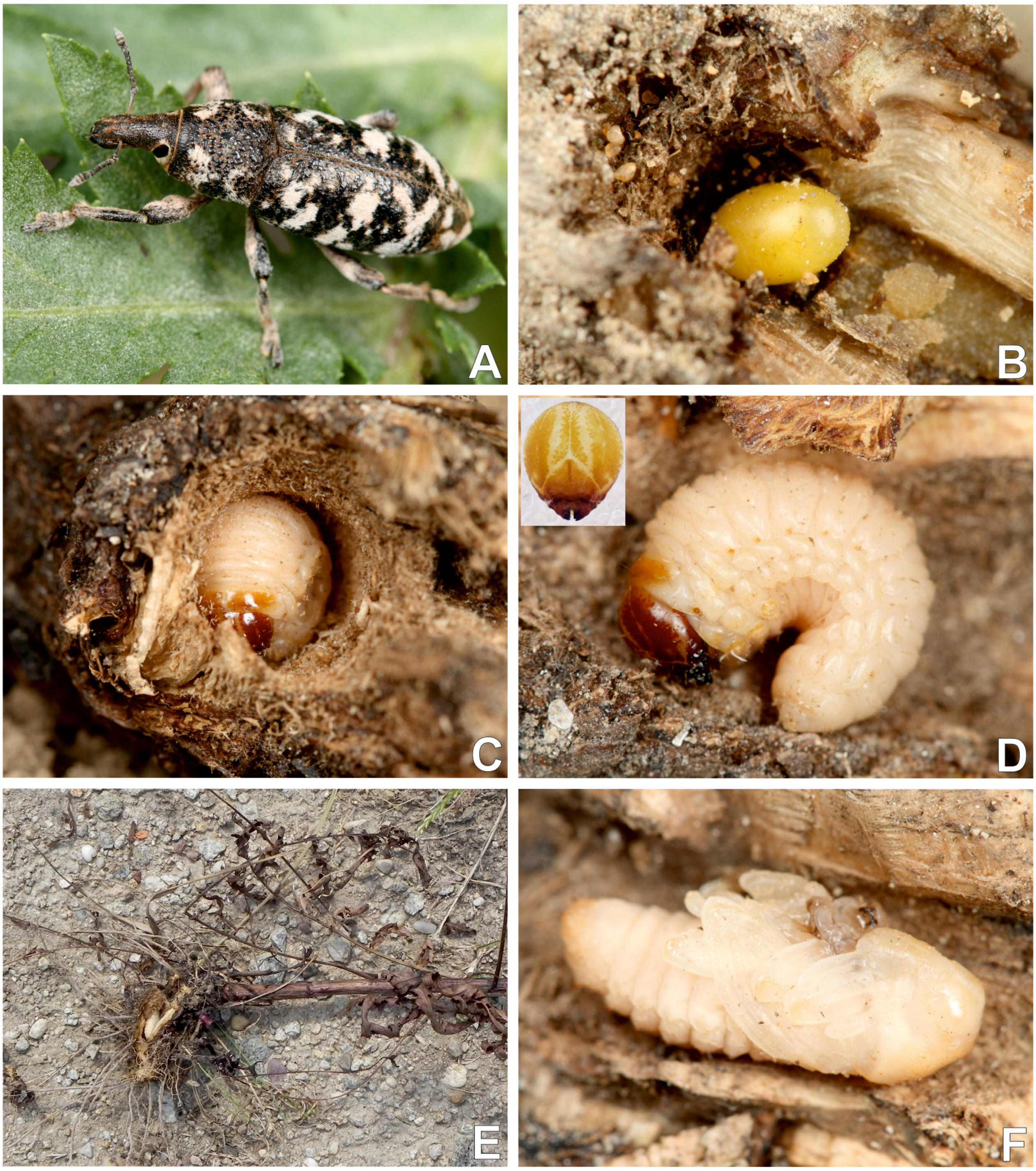

FIGURE 4. Adult and immature stages of *Adosomus roridus* (Pallas, 1781): A—adult, B—egg in roots of *Tanacetum vulgare*, C—mature larva in gall, D—mature larva (removed from gall), E—pupa in gall of *Tanacetum vulgare*, F—pupa (removed from gall).

Tanacetum vulgare and *Artemisia vulgaris* are native to Europe and Asia and are considered invasive species in parts of North America (Grulich 2004, Zelený 2004). *T. vulgare* is an archaeophyte in central Europe, originating from the Mediterranean region. In our opinion, using the weevil *A. roridus* as a biological control agent would be less effective and potentially dangerous. The main problem with this species is its oligophagy. Introducing and using *A. roridus* as a biological control agent might be risky for indigenous fauna/flora, similar to introduction of the weevil *Rhinocyllus conicus* (Louda *et al.* 2003). The latter was introduced to North America to suppress invasive European *Carduus* and *Cirsium* species. Unfortunately, *R. conicus* strongly invaded the targeted plants and eventually native species, namely, wavyleaf thistle (*Cirsium undulatum*). The wavyleaf thistle is a preferred host of a native tephritid fly, *Paracantha culta* (Wiedemann, 1830). The larvae of this fly develop in flowerheads, similar to larvae of *R. conicus*, so its populations are often negatively influenced by *R. conicus* (Louda *et al.* 2003). Individuals of oligophagous *A. roridus* might prefer local plants of the *Tanacetum*, *Artemisia* and other related genera, resulting in risks for indigenous flora and fauna. Before we can use *A. roridus* as a biological control agent, we have to know the connections between its host plants (e.g., nutrients, tannins, etc.).

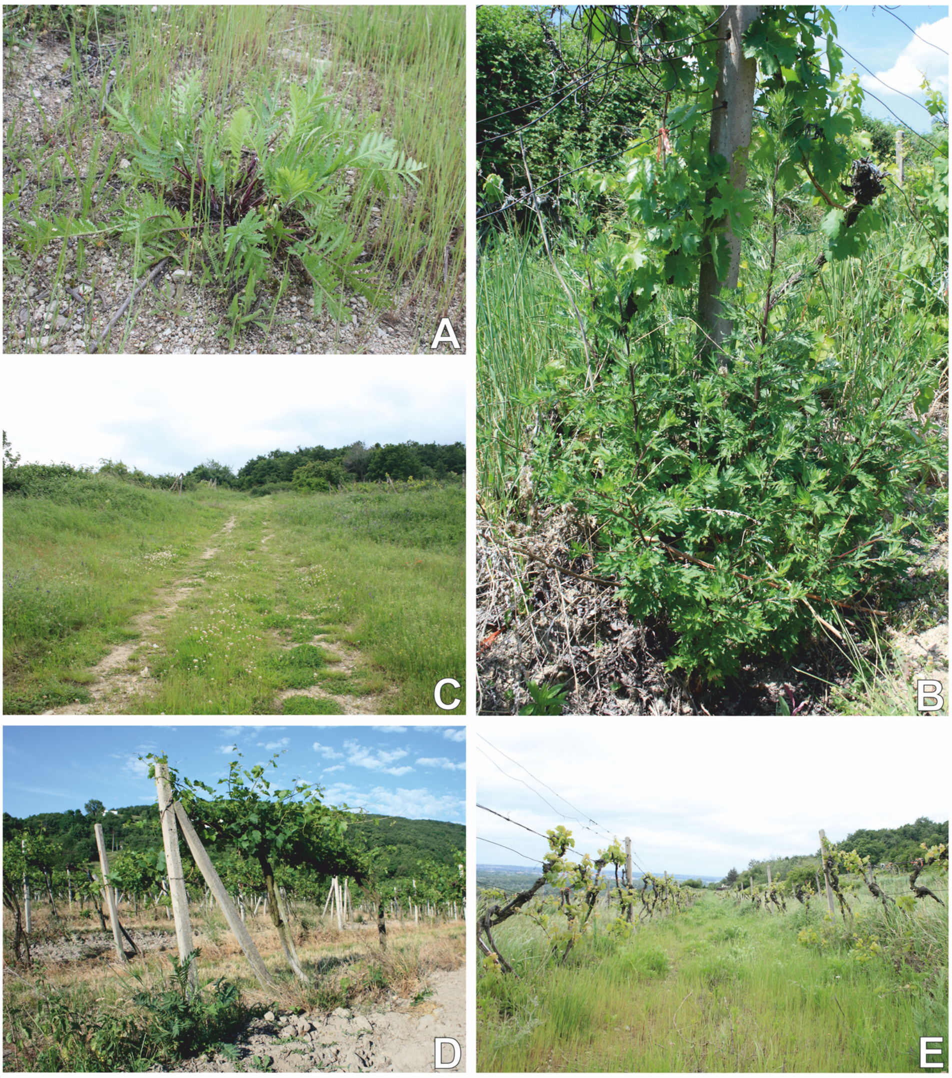

FIGURE 5. Host plants and habitats of *Adosomus roridus* (Pallas, 1781): A—young host plant of *Tanacetum vulgare*, B—host plant *Artemisia vulgaris*, C–E: habitat in Slovakia (Pezinok-Grinava).

The bionomy of adult individuals is of great interest because the majority of the central-European Cleonini species requires a specific regime of habitat disturbance, resulting in sparse vegetation cover (Stejskal & Trnka 2013, 2014). *Adosomus roridus* was specifically found at disturbed places with sparse vegetation but, in contrast, also at places with very dense, unbroken vegetation. This is in accordance with observations of some previous authors (e.g. Roubal (1937–1941) who mentioned "rich vegetation with *Artemisia*, *Tanacetum*, *Rubus* and *Calamagrostis*" as habitat of *A. roridus*. Similar habitat preferences and the same host plants are only known for *Cyphocleonus dealbatus* (Gmelin, 1790) in central Europe (Stejskal & Trnka unpubl.) which we collected in the studied locality as well. In addition to vineyards and ruderal vegetation, as presented in our study, *A. roridus* was recorded in a wide range of habitats including xerothermic grasslands of associations from the class *Festuco-Brometea* (Mazur 2002), sand dunes and salt marshes (Majzlan 1995), xerothermic forest-steppe associations of the Danubian forest steppe (*Asparago-Crataegetum danubiale*) (Majzlan 1990 (as *Crataegetum danubiale*), Šimonovičová *et al.* 2008) and oak forests (Roubal 1937–1941).

Adults of *A. roridus* probably do not move much because they are commonly found under stones (Kofler 1962–1963) or plant rosettes. In addition, they are not trapped in pitfalls (C. Germann, in litt), which may also imply their low mobility.

Adosomus roridus is often found in low altitudes in Slovakia (Kodada & Majzlan 1991, Majzlan 1995, Majzlan 2007), as confirmed by our findings (175 m a.s.l.), although this weevil seems ambivalent towards altitude. Some findings from Switzerland show that *A. roridus* appears at up to 1700 m a.s.l., where it inhabits the slopes of southern exposure with montane to subalpine xerothermic grasslands (C. Germann, in litt). Furthermore, preference for a geological base is not strict. Our findings come from the Quaternary sediments (sandy gravels), which is a similar geological base to other localities of *A. roridus* in Slovakia (e.g., Kodada & Majzlan 1991, Majzlan 2007). However, from Switzerland, we have findings from chalkstone/limestone or granite (C. Germann, in litt).

Overwintering of larvae and adult individuals is different from the other Cleonini species. Only mature larvae or adult individuals of the other Cleonini species overwinter. With regard to *A. roridus*, different age stages of larvae overwinter together with the adult specimens, which is unusual for Cleonini. Larvae mature from May, but adult weevils can be found from early spring (overwintered adults) to autumn (adults of new generation).

Knowledge of immature stages and life histories can help to protect endangered species (including the species presented here) more effectively. The detailed description of the larva and pupa, and its comparison with known descriptions reported here, demonstrates the possibility of identifying this species in its immature stages. Unfortunately, the relatively low number of available descriptions in comparison to the great number of known adult weevil species is a problem in the taxonomic use of the immature stages, as has been done by other groups (Hyperini: Skuhrovec 2007, Entiminae: Gosik & Sprick 2012a, b, 2013, Curculioninae, Tychiini: Skuhrovec *et al.* 2014, 2015). Currently, key and detailed generic studies of the Cleonini are impossible because of our limited knowledge of immature stages. The main problem is that we are unable to divide the morphological characteristics in this group into (i) characteristics useful for phylogenetics and (ii) characteristics useful for species identification. Once this categorisation is complete, it will be possible to use it for future phylogenetic studies.

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PŘÍLOHA IV

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The morphology of the immature stages of two rare *Lixus* species (Coleoptera, Curculionidae, Lixinae) and notes on their biology

Filip Trnka[1], Robert Stejskal[2], Jiří Skuhrovec[3]

[1] Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, CZ-783 71 Olomouc, Czech Republic **2** *Administration of Podyji National Park, Na Vyhlídce 5, CZ-669 02 Znojmo, Czech Republic* **3** *Group Function of Invertebrate and Plant Biodiversity in Agrosystems, Crop Research Institute, Drnovská 507, CZ-161 06 Praha 6 – Ruzyně, Czech Republic*

Corresponding author: *Filip Trnka* (filip.trnka88@gmail.com)

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Abstract

The mature larvae and pupae of *Lixus* (*Ortholixus*) *bituberculatus* Smreczyński, 1968 and *L.* (*Dilixellus*) *neglectus* Fremuth, 1983 (Curculionidae: Lixinae: Lixini) are described and compared with known larvae of 21 other *Lixus* and 2 *Hypolixus* taxa. The mature larva and pupa of *L. bituberculatus* are the first immature stages described representing the subgenus *Ortholixus*. The larva of *L. neglectus*, in the subgenus *Dilixellus*, is distinguished from the known larvae of four species in this subgenus by having more pigmented sclerites on the larval body. All descriptions of mature larvae from the tribe Lixini, as do all known species from the tribe Cleonini, fit the diagnosis of the mature larva of the Lixinae subfamily. Furthermore, new biological information of these species in the Czech Republic, Slovakia and Romania is provided. For *L. bituberculatus,* a chicory, *Cichorium intybus* L. (Asteraceae), is identified as a host plant, and *L. neglectus* is found on dock *Rumex thyrsiflorus* Fingerh. (Polygonaceae). Both species are probably monophagous or oligophagous. Adults of *L. bituberculatus* often inhabit host plants growing in active, dry and sunny pastures with sparse patches without vegetation, being mostly active during the night in April/May and then again in September, when the highest activity levels are observed. Adults of *L. neglectus* inhabit dry grasslands on sandy soils with host plants, being active during the day from May to September, with the highest level of activity in May/June and September. The larvae of both species are borers in the stem and root of the host plant, and they pupate in root or root neck. Adults leave the pupation cells at the end of summer and do not hibernate in the host plants. Finally, Romania is a new geographic record for *L. bituberculatus*.

Keywords

Weevil, mature larva, pupa, larval development, life cycle, host plant, *Cichorium intybus*, *Rumex thyrsiflorus*, Central Europe, Palaearctic region

Introduction

The genus *Lixus* Fabricius, 1801, belongs to the tribe Lixini Schoenherr, 1823 in the subfamily Lixinae Schoenherr, 1823 (family Curculionidae Latreille, 1802), and approximately 500 species have been described globally (Ter-Minasian 1967). Approximately 170 *Lixus* species in 12 subgenera are found in the Palaearctic region; only *Eutulomatus* Desbrochers des Loges, 1893 and *Parileomus* Voss, 1939 do not occur in Europe (Gültekin and Fremuth 2013). The biology of these species is partially known but has primarily only been studied recently (e.g., Nikulina 1989; Volovnik 1994, 2007; Gültekin 2007; Gosik and Wanat 2014; Skuhrovec and Volovnik 2015). The immature stages of *Lixus* species usually develop in the stems (Meregalli 2014) or the roots of plants (Dieckmann 1983) but sporadically develop in the seed capsule (Gültekin 2005) or petiole (Gültekin 2007). Some species from this genus are considered to be agricultural pests (e.g., *Lixus incanescens* Boheman, 1835 in Manole 1990; others in Volovnik 1988; Nikulina 1989), but others could be used for the biological control of selected weeds (e.g., *Lixus filiformis* (Fabricius, 1781) for musk thistle; Gültekin 2004) or have already been used for these purposes (e.g., *Lixus cardui* Olivier, 1807 in Australia; Nikulina and Gültekin 2011). Detailed morphological descriptions have been published for the larvae of 21 *Lixus* species, with detailed descriptions of the pupae being available for only eight species (see Scherf 1964; Lee and Morimoto 1988; May 1994; Nikulina 2001, 2007; Zotov 2009a, b; Nikulina and Gültekin 2011; Gosik and Wanat 2014; Skuhrovec and Volovnik 2015).

Lixus bituberculatus Smreczyński, 1968 belongs to the subgenus *Ortholixus* Reitter, 1916, which includes 18 species in the Palaearctic region (Gültekin and Fremuth 2013). This species is among the rarest of the genus *Lixus*, only distributed in Hungary, Slovakia and Bulgaria (Gültekin and Fremuth 2013; Stejskal and Trnka 2014). The biology and host plant of *L. bituberculatus* were completely unknown, and its immature stages have never been described.

Lixus neglectus Fremuth, 1983 belongs to the subgenus *Dilixellus* Reitter, 1916, which includes 31 species in the Palaearctic region (Gültekin and Fremuth 2013), and the distribution of this central European endemic weevil includes a relatively small area (ca. 5000 km^2) in Austria, the Czech Republic and Slovakia at the confluence of the Dyje and Morava Rivers (Gültekin and Fremuth 2013; Trnka and Stejskal 2014). The biology and plant associations of *L. neglectus* are better known than previously described species, and several authors reported the occurrence of adults on garden sorrel (*Rumex acetosa* L.) (e.g., Fremuth 1983; Koch 1992; Böhme 2001; Trnka and Stejskal 2014). The immature stages of *L. neglectus* have never been described.

Knowledge of the immature stages and life histories of both species is important for taxonomy as well as practical applications and can help to more effectively protect these species. In this paper, we describe the immature stages of both species and provide details of their life history based on field observations in the Czech Republic, Slovakia and Romania.

Materials and methods

The material used to describe the immature stages was collected, and field observations were conducted in the localities mentioned below:

Lixus (*Ortholixus*) *bituberculatus* Smreczyński, 1968

Material examined. ROMANIA: **Caraș-Severin County:** Sfânta Elena env.; 44°40'24.1"N, 21°43'2.0"E; survey dates: 9-VI-2012, 1–2-IX-2012, 18–23-V-2013, 5-VIII-2014 (3 larvae), 6-VIII-2014 (2 larvae, 1 pupa), 8-VIII-2014 (7 larvae, 3 pupae); all leg. & det. F. Trnka, coll. J. Skuhrovec. Habitats: pastures (cattle, sheep, goats), road margins and dry grasslands. Bedrock: limestone. Altitude: 400 m a. s. l. (see Fig. 21). **SLOVAKIA**: **Rimavská Sobota District:** Gemerské Dechtáre env.; 48°15'22.03"N, 20°2'12.44"E; 11-IV-2015. Habitats: pastures (cattle and sheep), road margins and dry grasslands. Bedrock: quaternary eolithic sediments (sand and loess). Altitude: 206 m a. s. l.

Lixus (*Dilixellus*) *neglectus* Fremuth, 1983

Material examined. CZECH REPUBLIC: **Břeclav District:** Lanžhot env.; 48°41'21.04"N, 16°56'3.40"E; survey date: 19-VII-2014. Habitat: dry grassland and blown sand, THG01 *Potentillo heptaphyllae-Festucetum rupicolae* (Chytrý et al. 2010). Bedrock: quaternary alluvial sediments (sand). Altitude: 164 m a. s. l. (see Fig. 41); Kostice env.; 48°45'54.60"N, 16°56'36.54"E; survey date: 9-VI-2015. Habitat: grassy road embankment. Bedrock: artificial structure (sandy gravel). Altitude: 168 m a. s. l. **SLOVAKIA**: **Trnava District:** Sekule env.; 48°37'8.60"N, 16°59'21.03"E; survey dates: 13-VII-2014 (5 mature larvae, 2 younger larvae, 1 pupa) and 25-VII-2014 (2 pupae reared from larvae collected on 13-VII-2014); all leg. R. Stejskal & F. Trnka, det. R. Stejskal & F. Trnka, coll. J. Skuhrovec. Habitat: dry meadow. Bedrock: quaternary alluvial sediments (sand). Altitude: 158 m a. s. l.

Rearing and life cycle observations were conducted during the 2014–2015 vegetation growing seasons. Laboratory observations were conducted in Olomouc (49°35'36"N, 17°15'3"E) and in Znojmo, Czech Republic (48°51'31"N, 16°2'40"E).

Part of the larval and pupal material was preserved in Pampel fixation liquid (4 parts glacial acetic acid, 6 parts 4% formaldehyde, 15 parts 95% ethyl alcohol and 30 parts distilled water) and used for the morphological descriptions. These specimens are now deposited in the Group Function of Invertebrate and Plant Biodiversity in Agrosystems of the Crop Research Institute (Prague, Czech Republic). Plants were identified by the collectors. Slides were prepared following May (1994) as follows: a larva was decapitated, its head was cleared in a 10% potassium hydroxide (KOH) solution and then rinsed in distilled water. After clearing, the mouth parts were separated from the head capsule. The head capsule and the mouth parts were mounted on permanent microscope slides in Euparal. The body parts (thorax and abdomen) were mounted on temporary microscope slides in 10% glycerine.

The observations and measurements were made using a light microscope with calibrated oculars (Olympus BX 40 and Nikon Eclipse 80i), and the following characteristics were measured for each larva: head width, length of the body (larvae fixed in a C-shape were measured in segments), width of the widest part of the body (metathorax or abdominal segments I–IV).The length and width of the widest part of the body was measured for each pupa. The thorax and abdomen were not sclerotised, and it is unlikely that the fixation process altered the weevils' proportions; measurements of these parts are given for comparison purposes only.

Drawings were made with a drawing tube on a light microscope and processed by a computer program (Adobe Photoshop, Corel Photo-Paint 11, GIMP 2). The thoracic spiracle is located on the prothorax near the boundary of the prothorax and mesothorax, as shown in the drawing (see Figs 8, 29), but it is of mesothoracic origin (Marvaldi et al. 2002, Marvaldi 2003). The drawings show the thoracic and abdominal spiracles (see Figs 8–10, 29–31). The numbers of setae are given for one side of the bilateral structures.

We used the terms and abbreviations for the setae of the mature larva and pupa studied in Scherf (1964), May (1977, 1994) and Marvaldi (1998a, 1999).

The count of some of the setae on the epipharynx (especially *ams* and *mes*) have not been completely resolved. According to Marvaldi (1998a, 1999), the standard status of the epipharynx in weevils is 2 *ams* and 3 *mes*, but when the position of the distal *mes* is very close to the anterior margin, they appear as *ams*. The decision was finally made to add this problematic seta to the latter group (*ams*), and the position of this seta is similar to that in other genera, e.g., in *Coniocleonus* Motschulsky or *Tychius* Germar. We did not follow Stejskal et al. (2014) and Skuhrovec et al. (2014), who accepted the standard status in weevils and counted the seta as *mes*, but we followed Trnka et al. (2015) and Skuhrovec et al. (2015), e.g., in *Adosomus* Faust or *Sibinia* Germar.

Results

Lixus (*Ortholixus*) *bituberculatus* Smreczyński, 1968

Description of mature larva. *Measurements* (in mm). Body length: 6.5–10.4 (mean 9.2). The widest part of the body (metathorax and abdominal segments I–II) measuring up to 2.8. Head width: 1.4–1.7 (mean 1.5).

General. Body stocky, slightly curved, rounded in cross section (Fig. 7). Cuticle finely spiculate.

Colouration. Head light brown or brown with a distinct pale pattern around the frontal suture (Figs 7, 19). All thoracic and abdominal segments are white with a light brown, elongate stripe on the dorsum of the pronotum (Fig. 7).

Vestiture. Setae on body thin, relatively short, light yellow or orange.

Head capsule (Fig. 1). Head suboval, flattened laterally, endocarinal line long more than half length of frons. Frontal sutures distinct, extended to the antennae. Single stemma (st) in the form of a slightly pigmented spot located anterolaterally on each side. *Des1* and *des2* located in the upper part of the central part of the epicranium, *des1* near the middle part of epicranium, and *des2* near the side of the epicranium, *des3* located anteriorly near the frontal suture, *des4* located in the central part of the epicranium, *des5* located anterolaterally; all *des* long, subequal in length (Fig. 1). *Fs1* and *fs2* placed medially, *fs3* located anteromedially, *fs4* located anterolaterally, and *fs5* located laterally, close to the epistoma; all setae relatively long, *fs4* slightly longer than *fs1-3* and *fs5* distinctly longer than *fs4* (Fig. 1). *Les1–2* as long as *des1*; *ves1–2* as long as *fs3*. Epicranial area with sensilla undistinct.

Antennae located at the end of the frontal suture on each side, membranous and slightly convex basal article bearing one conical triangular sensorium, relatively long; basal membranous article with 5 sensilla different in both shape and length (Fig. 4).

Clypeus (Fig. 2) approximately 2.1 times as wide as long with 2 relatively long *cls*, almost equal in length, localized posterolaterally and 1 sensillum; anterior margin rounded to the inside.

Mouth parts. Labrum (Fig. 2) approximately 2 times as wide as long, with 3 pairs of piliform *lms*, of different lengths; *lms3* distinctly shorter than very long *lms1* and long *lms2*; *lms1* located close to the margin with clypeus, *lms2* located anteromedially and *lms3* located anterolaterally; anterior margin double sinuate. Epipharynx (Fig. 3) with 4 pairs of blunt, finger-like *als*, unequal in length, *als1–2* distinctly shorter than *als3–4*; 3 pairs of *ams*, *ams1* and *ams2* distinctly shorter than *ams3*, *ams1* and *ams2* piliform, and *ams3* blunt, finger-like; 2 pairs of short, blunt *mes* and one sensilla close to *mes2*, located close to lr; labral rods (lr) elongated, converging anteriorly. Mandibles (Fig. 5) relatively broad, bifid, teeth of unequal height; slightly truncate; both *mds* relatively long, piliform. Maxilla (Fig. 6) stipes with 1 *stps*, 2 *pfs* and 1 *mbs*; very long *stps* distinctly longer than long *pfs1–2*, *mbs* very short; mala with 12 bacilliform *dms* of two different lengths (6 very long and 6 relatively long); 5 short *vms*, almost equal in length; *vms* distinctly shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with 1 very short *mxps* and two sensilla; length ratio of basal and distal palpomeres: 1:0.7; distal palpomere with one sensillum and a group of conical, apical sensorial papillae. Praelabium (Fig. 6) heart-shaped and distinctly elongated, with 1 relatively long *prms*; ligula with sinuate margin and 3 piliform micro *ligs*, unequal in length; premental sclerite well visible. Labial palpi with two palpomeres; length ratio of basal and distal palpomeres: 1:0.7; distal palpomere with one sensillum and short,

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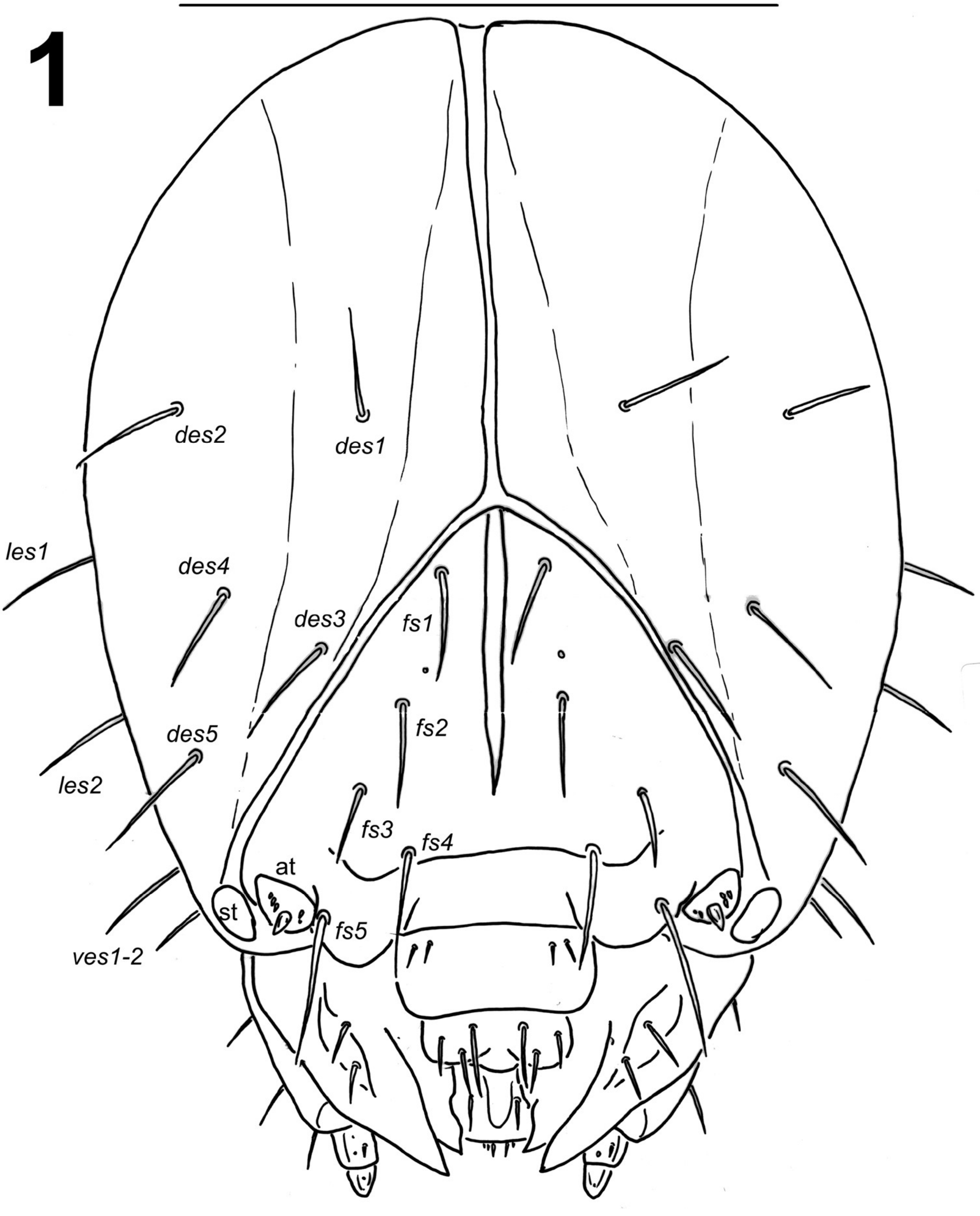
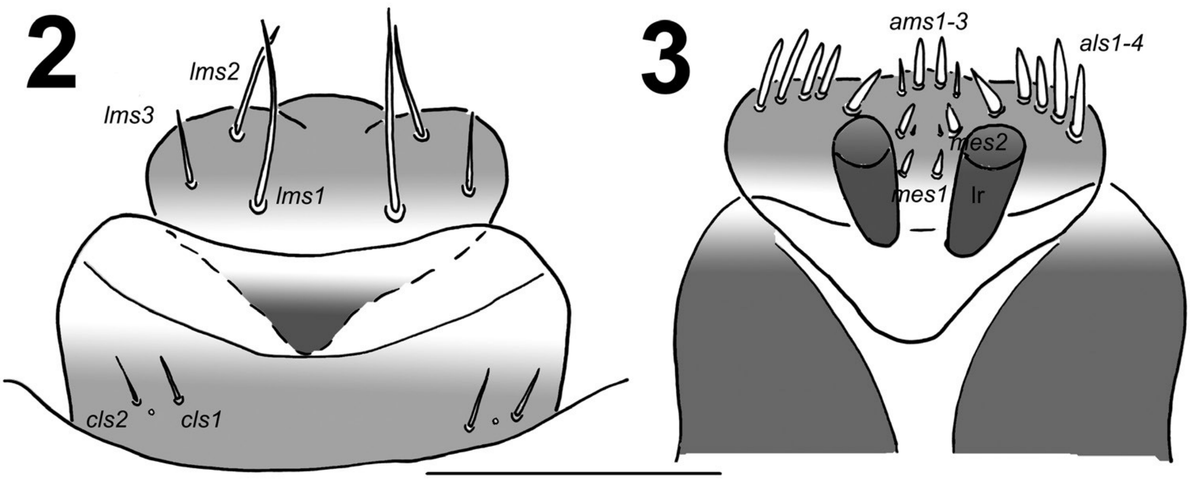


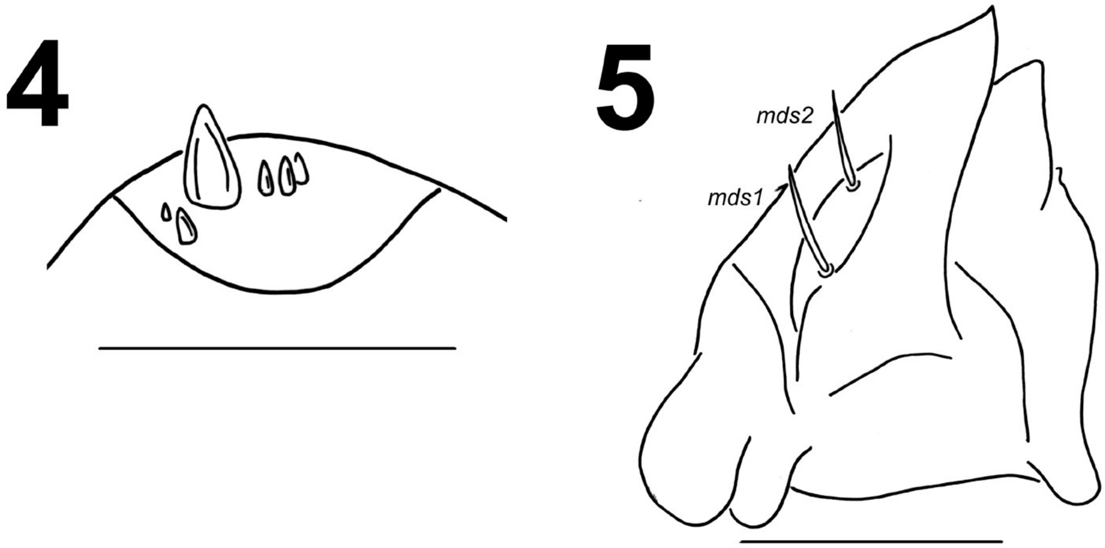
Figure 1. *Lixus bituberculatus* mature larva head, dorsal view. Scale bar: 1 mm.

apical sensorial papillae; basal palpomere with 1 ventral sensillum. Postlabium (Fig. 6) with 3 *pms*, *pms1* located anteriorly, remaining two pairs laterally; relatively long, almost of equal length, *pms3* distinctly shorter than *pms1* and *pms2*; surface of postlabium densely covered by distinct asperities.

Thorax. Prothorax distinctly smaller than meso- and metathorax. Metathorax almost of equal length as abdominal segments I–IV. Spiracle bicameral. Prothorax



Figures 2–3. *Lixus bituberculatus* mature larva. **2** Labrum and clypeus **3** Epipharynx. Scale bar: 0.5 mm.



Figures 4–5. *Lixus bituberculatus* mature larva head. **4** Antenna **5** Right mandible. Scale bars: 0.1 mm (**4**) and 0.2 mm (**5**).

(Fig. 8) with 10 *prns* unequal in length, 8 of them on weakly pigmented premental sclerite, which is subdivided medially into two triangular plates, next two *prns* placed below; 2 *ps* and 2 *eus*. Mesothorax (Fig. 8) with 1 *prs*; 4 *pds* unequal in length, *pds2* distinctly shorter than the remaining three setae; 1 short *as*; 2 short to very short *ss*; 1 *eps*; 1 *ps* and 2 *eus*. Chaetotaxy of metathorax (Fig. 8) identical to that of mesothorax. Each pedal area of the thoracic segments well separated and pigmented, with 7 long *pda*, 6 of which on pigmented area, unequal in length.

Abdomen. Abdominal segments I–IV of almost equal length, subsequent abdominal segments decreasing gradually to the terminal parts of the body. Abdominal segment X reduced to four anal lobes of unequal size, the dorsal being distinctly the largest, the lateral pair equal in size, and the ventral lobe very small. Anus located terminally. Spiracles bicameral, the eight abdominal spiracles located laterally, close to

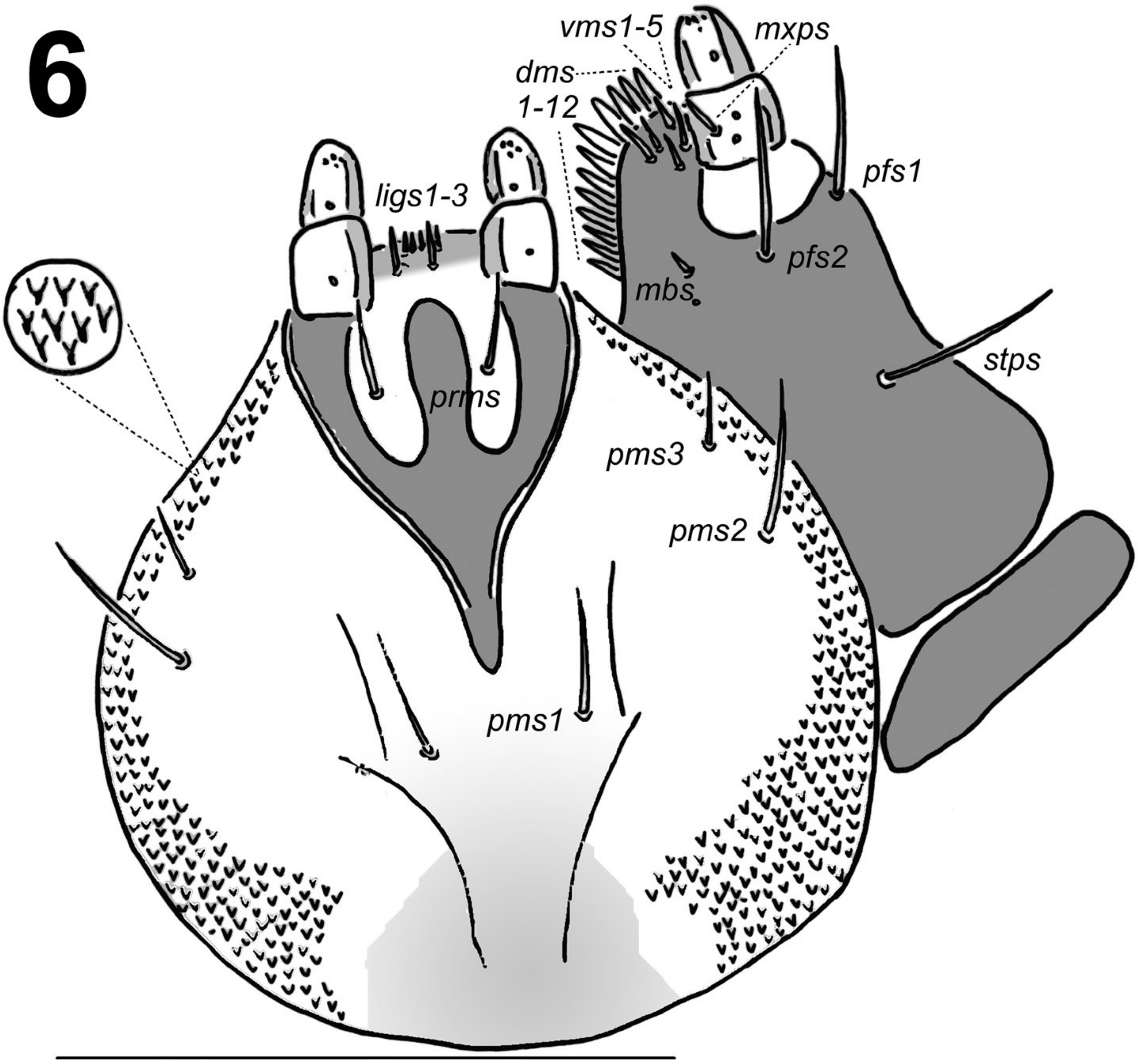


Figure 6. *Lixus bituberculatus* mature larva head, maxillo-labial complex, ventral view. Scale bar: 0.5 mm.

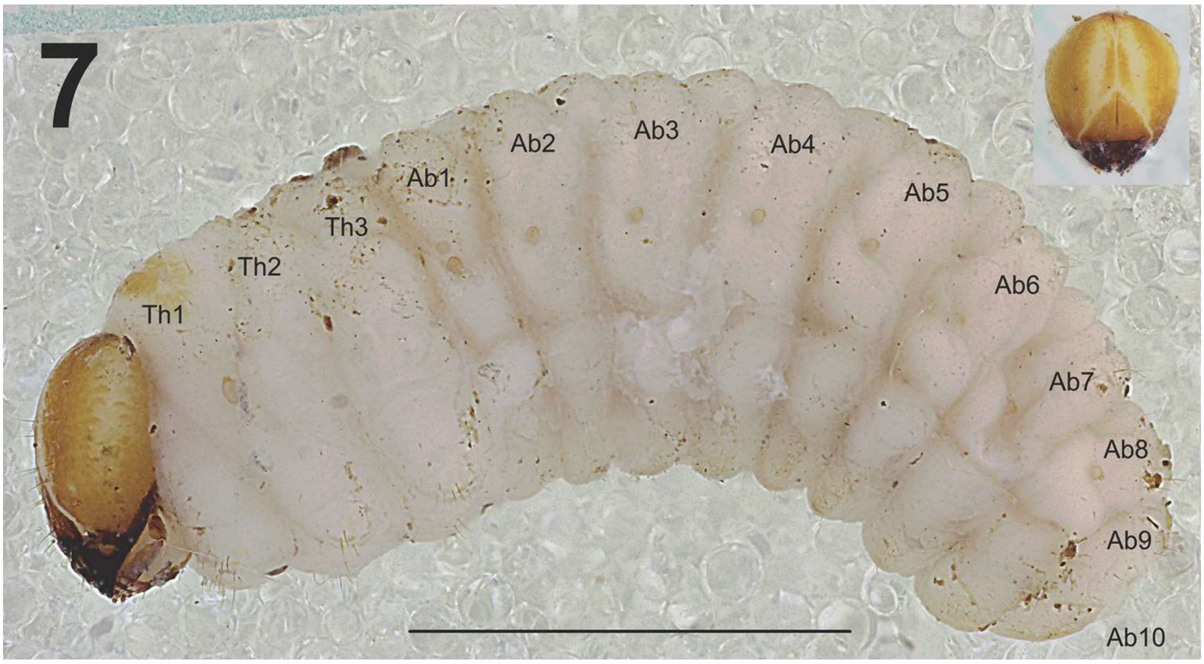
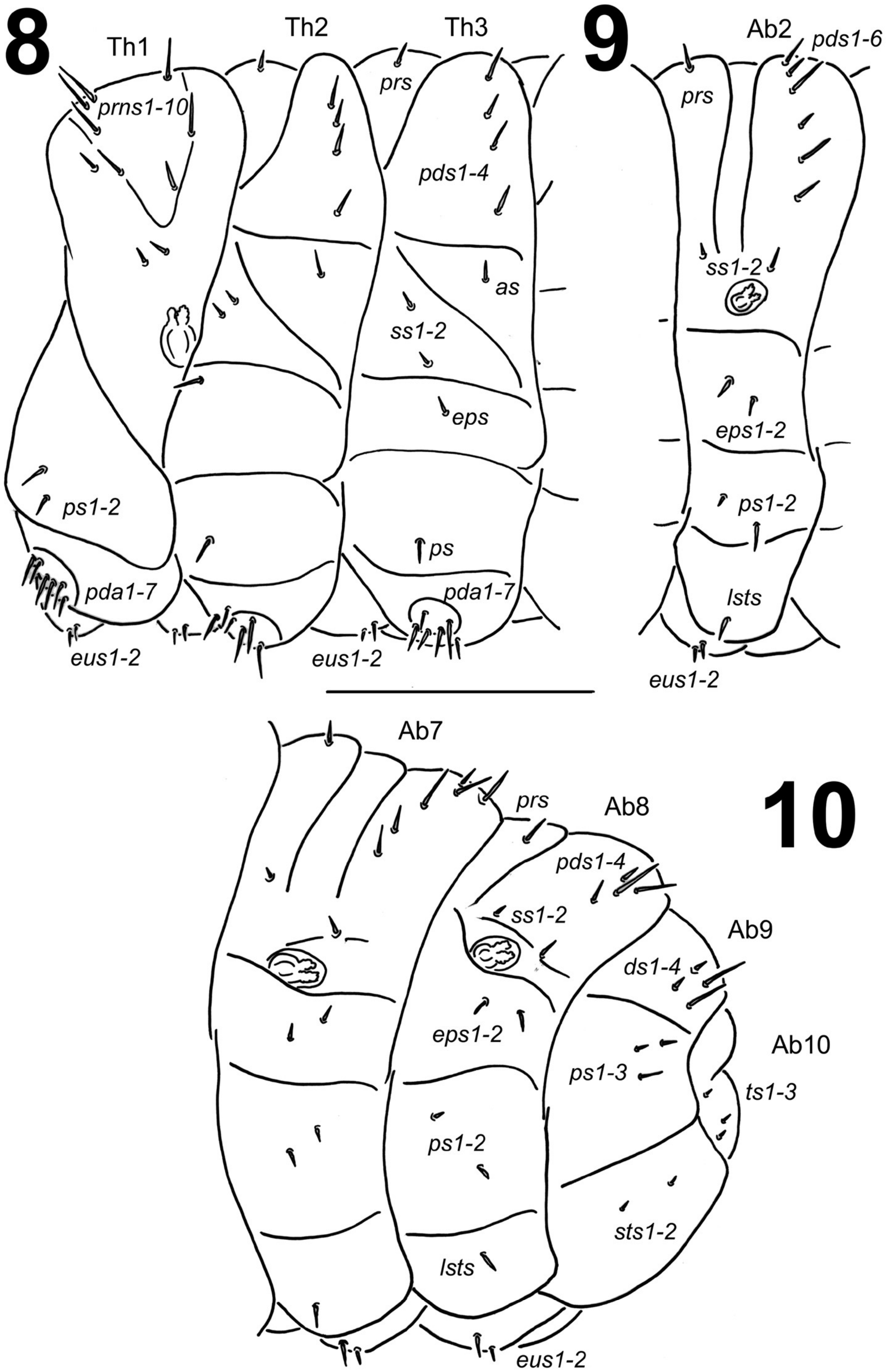


Figure 7. *Lixus bituberculatus* mature larva habitus, lateral view. Scale bar: 3 mm.



Figures 8–10. *Lixus bituberculatus* mature larva habitus. **8** Lateral view of thoracic segments **9** Lateral view of abdominal segment II. **10** Lateral view of abdominal segments VII–X. Scale bar: 1 mm.

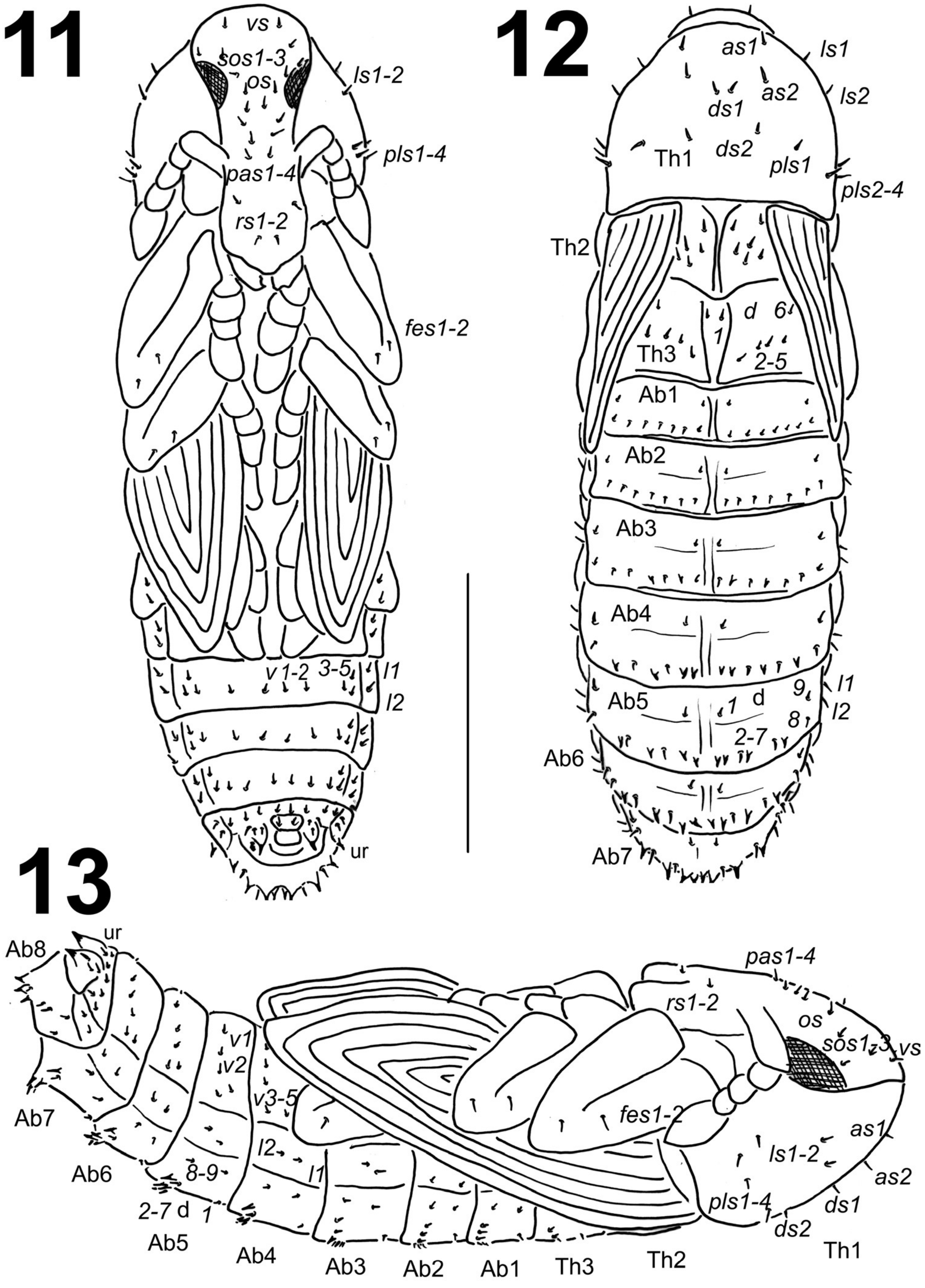
the anterior margin of abdominal segments I–VIII. Abdominal segments I–VII (Figs 9–10) with 1 *prs*; 6 *pds*, *pds3* and *pds5* the longest one; 2 *ss* of unequal length, *ss1* very short, *ss2* as long as *pds6*; 2 *eps* of almost equal length; 2 relatively short *ps* of unequal length, *ps1* very short to minute, *ps2* short; 1 *lsts* and 2 *eus*. Abdominal segment VIII (Fig. 10) with 1 *prs*; 4 *pds*, *pds1* and *pds6* lacking, *pds3* and *pds5* less than half of length of the two remaining setae; 2 *ss* of unequal length, *ss1* very short, *ss2* long as *pds6*; 2 *eps* of almost equal length; 2 short *ps* of equal length; 1 *lsts* and 2 *eus*. Abdominal segment IX (Fig. 10) with 4 *ds* (*ds1* and *ds3* very short, *ds 2* and *ds4* long); 2 short and 1 very short *ps* and 2 very short to micro *sts*. Abdominal segment X (Fig. 10) with 2 microsetae and 1 seta (*ts*) on each lateral anal lobe.

Description of pupa. *Measurements* (in mm). Body length: 8.0–10.4 (♂ 8.0–10.4; ♀ 9.8). The widest part of the body, commonly between the apex of the meso- or metafemora: 2.6–3.5.

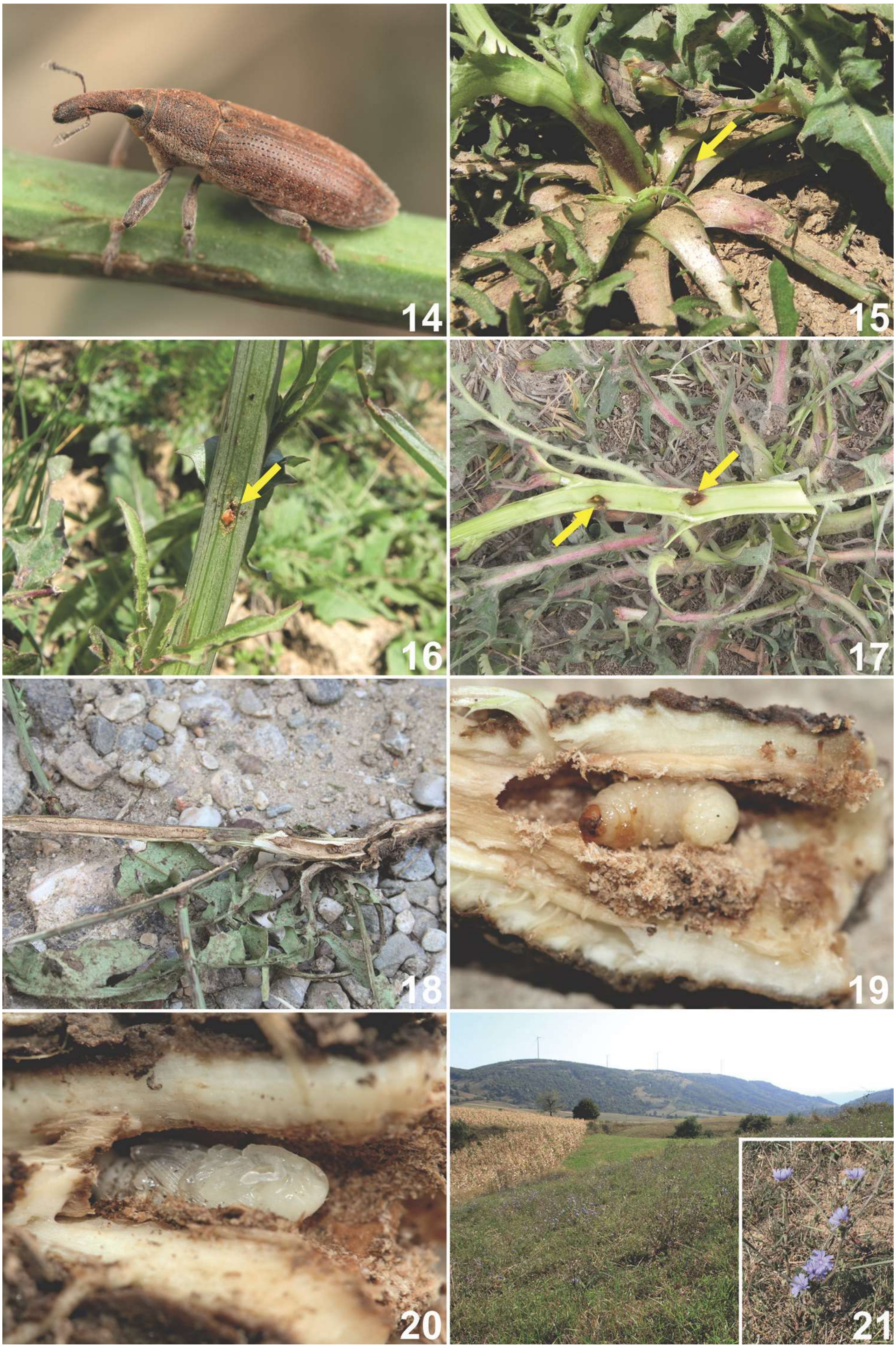
Colouration. Body white to yellowish (Fig. 20).

Morphology (Figs 11–13, 20). Body stocky, elongated, white or yellowish. Cuticle smooth. Rostrum relatively long, approximately 2.7 to 3.0 times as long as wide, extending beyond the mesocoxae; females with slightly thinner rostrum than males. Antennae relatively long and stout. Pronotum from 1.2 to 1.3 times as wide as long. Mesonotum and metanotum of almost equal length. Abdominal segments I–III of almost equal length; abdominal segment VI semicircular and subsequent abdominal segments diminish gradually to the end of the body. Abdominal segments VII–IX distinctly smaller than other abdominal segments. Gonotheca (abdominal segment IX) in females (1 specimen) bilobed.

Chaetotaxy (Figs 11–13). Setae relatively short, unequal in length, light yellow or orange, some setae on abdominal segments III–VIII distinctly stronger and located on protuberances. Setae well visible. Head capsule includes 1 *vs*, 3 *sos*, 1 *os* and 4 *pas*. Rostrum with 2 *rs*, *rs1* located below antenna, *rs2* placed on the anterior margin. Setae on head capsule and rostrum straight, both *rs* and all *pas* distinctly shorter than the remaining setae on head, thoracic and abdominal segments. Pronotum with 2 *as*, 2 *ds*, 2 *ls* and 4 *pls*. Dorsal parts of mesothorax with 1 seta located posteromedially, 1 seta located posterolaterally and 4 setae located along its anterior margin. Chaetotaxy of metathorax identical to that of mesothorax. Each femoral apex with 2 *fes*. Dorsal parts of abdominal segments I–VIII each with 2 pairs of setae located posteriorly (*d1*, *d9*) and 7 pairs (*d2–8*) located along their anterior margins. Setae *d2–3, d5* and *d7* (on abdominal segments IV–VIII) short, thorn-like, located on protuberances; on abdominal segment III only setae *d3* and *d5*. Remaining setae short to very short, hair-like; all setae very short on abdominal segments I–II. Abdominal segments I–VII with groups of 2 lateral setae and 5 pairs of ventral setae. Dorsal part of abdominal segment VIII with 1 seta located posteriorly (*d9*) and 7 pairs (*d2–8*) located along its anterior margin; *d3, d5* and *d7* thorn-like, located on protuberances; remaining setae elongated. Abdominal segment VIII with groups of 2 lateral setae and 5 short ventral setae. Abdominal segment IX with 2 pairs of ventral microsetae and 1 pair of short, thin setae. Urogomphi elongated, triangular.



Figures 11–13. *Lixus bituberculatus* pupa habitus. **11** Ventral view **12** Dorsal view **13** Lateral view. Scale bar: 3 mm.



Figures 14–21. Habitats, adults, immature stages and life cycle of *Lixus bituberculatus*. **14** Adult **15** Adult hiding in host plant rosette **16** Ovipositional mark **17** Eggs in the host plant stem **18** Feeding marks in the stem **19** Mature larva in the root crown **20** Pupa and pupation cell **21** Habitat in Romania and *Cichorium intybus* host plant.

Biology and ecology. *Habitats.* Adults (Fig. 14) prefer dry and sunny habitats such as dry grasslands, meadows often with grazing or mowing (Fig. 21), and road margins with specific disturbance regimes (trampling by movement of cattle or vehicles, etc.).

Adult behaviour. During the day, adults stay among the rosette leaves of the host plant (Fig. 15) near the stem base. Adults were usually observed by sweeping the host plants at night. Data were collected from April to September with the exception of July. The maximum number of records occurred in late summer.

Host plants. Adults and larvae were observed feeding on chicory *Cichorium intybus* L. (Asteraceae), in the studied localities (Fig. 21). Nevertheless, J. Krátký and J. Pelikán (in litt.) also found adult *L. bituberculatus* on *Crepis* sp. and *Picris* sp. during night sweeping and a pupa in the root of *Picris* sp. in Slovakia (Hajnáčka env. and Bajtava env.).

Life cycle. Lixus bituberculatus is an univoltine species. Adults feed on leaves, but larval development occurs in the basal part of the stem and in the root (Figs 18–19). Females of *L. bituberculatus* bite the lower part of the stem of the host plant near the ground and lay one egg in the hole (Figs 16–17). Usually, only one larva was found to occupy a plant, but rarely, there were two (one in the stem and another in the root crown). Mature larvae were found from July to August. Pupation occurs in the root neck or root (Fig. 20), and fresh adults can be found (inside plants) from middle of August. The exit hole is situated in the upper part of the pupation cell. Adults do not hibernate in the host plants. Most likely, hibernation occurs in the leaf litter, among dry plant debris or in the topsoil.

Rearing of the larvae. For laboratory breeding, 10 mature larvae were collected on August 8th, 2014, but only three pupated under our laboratory conditions. The remaining seven larvae primarily died due to drying of the host plants. The first fresh adult hatched on September 12th and the other two on September 15th, 2014.

Lixus (*Dilixellus*) *neglectus* Fremuth, 1983

Description of mature larva. *Measurements* (in mm). Body length: 10.5–13.5 (mean 12.5). The widest part of the body (metathorax and abdominal segments I–II) measuring up to 3.3. Head width: 1.8–2.1 (mean 2.0).

General. Body stocky, slightly curved, rounded in cross section (Fig. 28), densely covered by distinct asperities (mainly dorsal and ventral parts). Cuticle finely spiculate.

Colouration. Head light brown or brown (Figs 28, 38). All thoracic and abdominal segments are white with a light brown elongate stripe on the dorsum of the pronotum (Fig. 28).

Vestiture. Setae on body thin, relatively long to very long, light yellow or orange.

Head capsule (Fig. 22). Head suboval, flattened laterally, endocarinal line long more than half length of frons. Frontal sutures distinct, extended to the antennae. Single stemma (st) in the form of a slightly pigmented spot, located anterolaterally on each

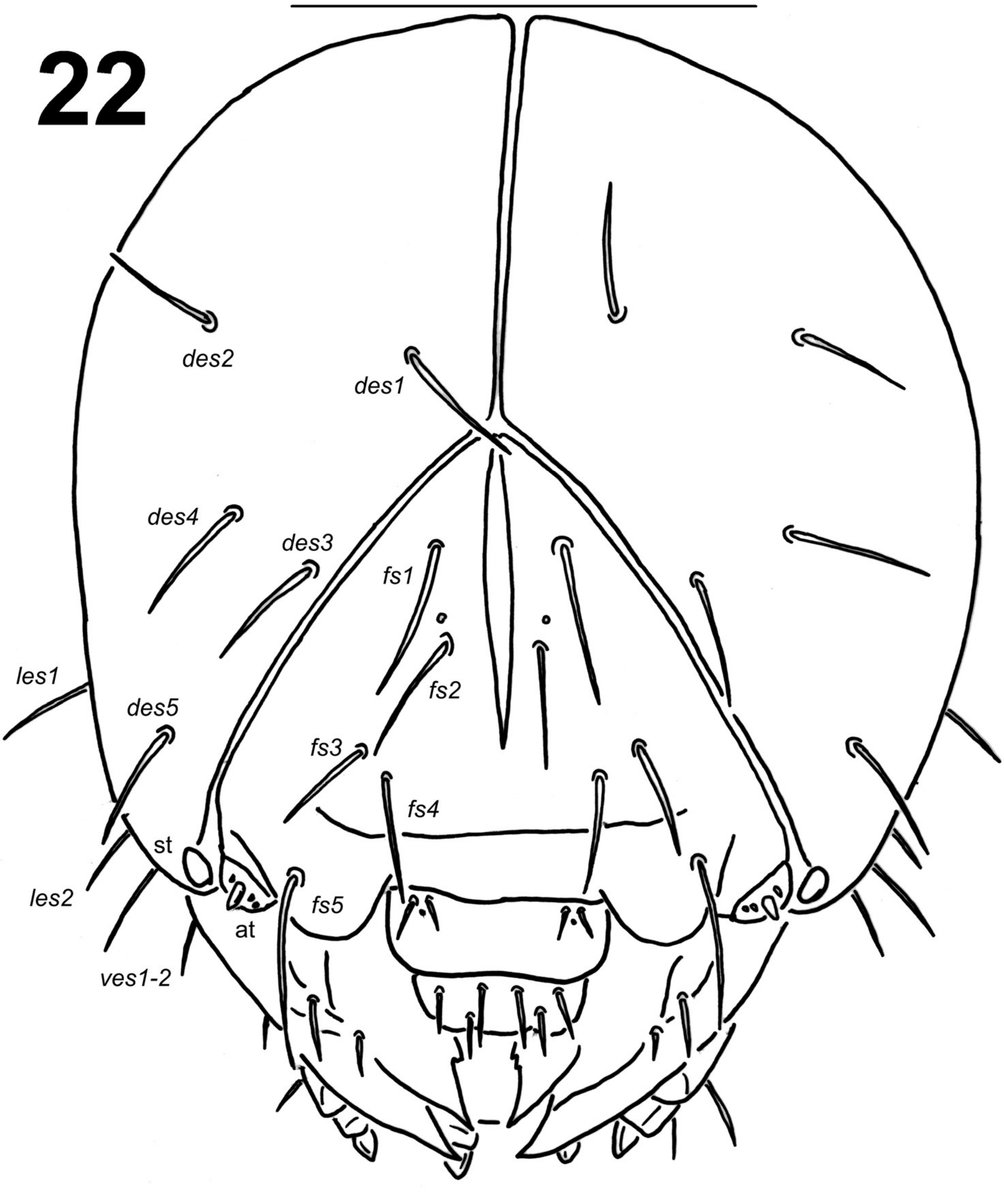


Figure 22. *Lixus neglectus* mature larva head, dorsal view. Scale bar: 1 mm.

side. *Des1* and *des2* located in the upper part of the central part of the epicranium, *des1* near the middle part of the epicranium, and *des2* near the side of the epicranium, *des3* located anteriorly near the frontal suture, *des4* located in the central part of the epicranium, *des5* located anterolaterally; all *des* long, subequal in length (Fig. 22). *Fs1* and *fs2* placed medially, *fs3* located anteromedially, *fs4* located anterolaterally, and *fs5* located laterally, close to the epistoma; all setae long to extremely long, *fs1* extremely long, *fs4* and *fs5* very long, distinctly longer than *fs2* and *fs3*, but shorter than *fs1* (Fig. 22). *Les1–2* as long as *des1*; *ves1–2* as long as *fs3*. Epicranial area with sensilla undistinct.

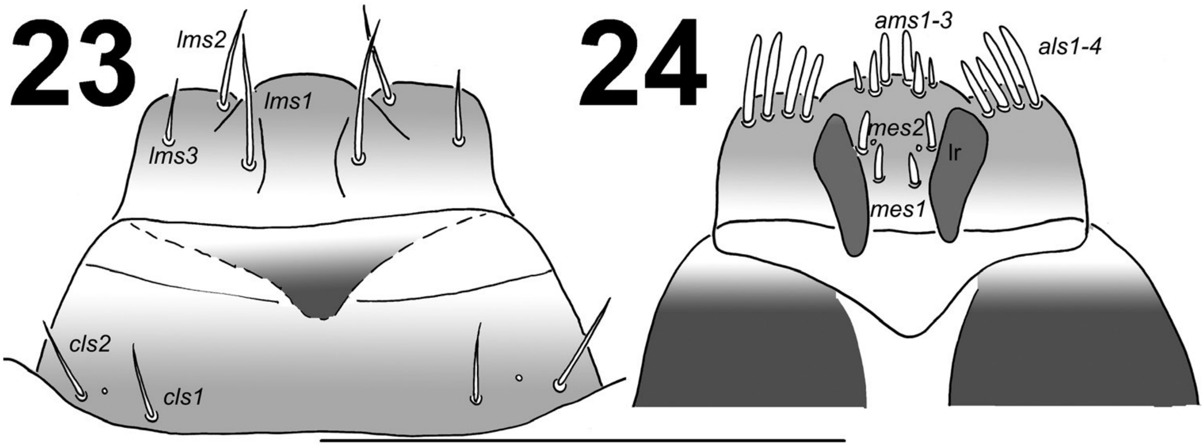
Antennae located at the end of the frontal suture on each side, membranous and slightly convex basal article bearing one conical triangular sensorium, relatively long; basal membranous article with 3 sensilla different in both shape and length (Fig. 25).

Clypeus (Fig. 23) approximately 2.5 times as wide as long with 2 long *cls*, almost equal in length, localized posterolaterally and 1 sensillum; anterior margin rounded to the inside; median part covered by thorn-shaped asperities.

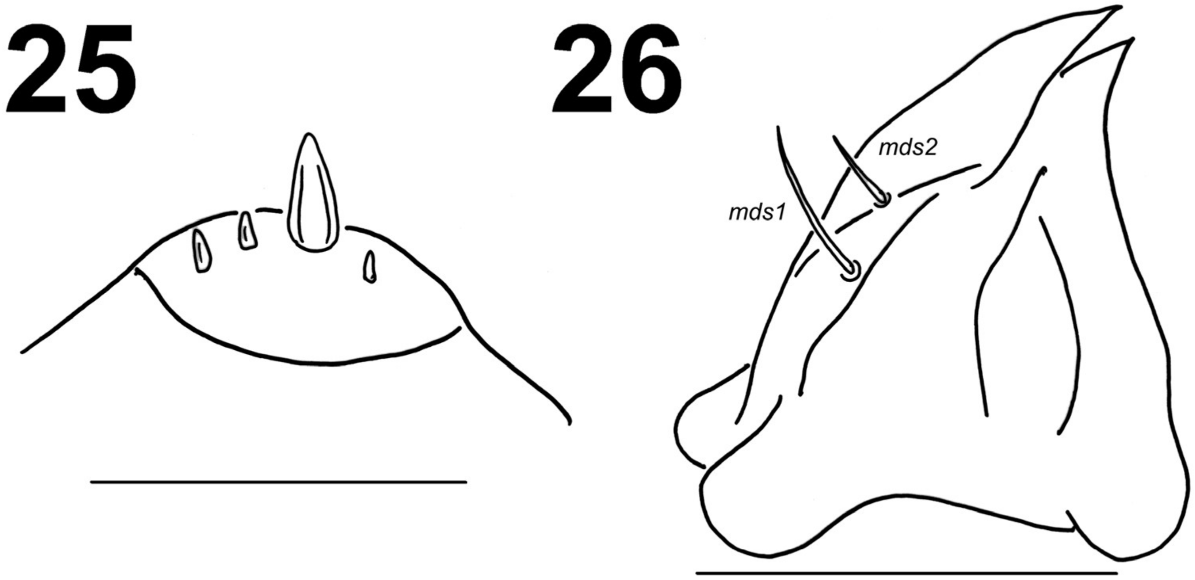
Mouth parts. Labrum (Fig. 23) approximately 3 times as wide as long, with 3 pairs of piliform *lms*, of different lengths; *lms3* distinctly shorter than very long *lms1* and long *lms2*; *lms1* placed close to the margin with clypeus, *lms2* located anteromedially and *lms3* located anterolaterally; anterior margin double sinuate. Epipharynx (Fig. 24) with 4 pairs of blunt, finger-like *als*, unequal in length, *als1–2* distinctly shorter than *als3–4*; 3 pairs of *ams*, *ams1* and *ams3* distinctly shorter than *ams2*, *ams1* and *ams3* piliform, and *ams2* blunt, finger-like; 2 pairs of short, blunt *mes* and one sensilla close to *mes2*; labral rods (lr) elongated, converging anteriorly, distinctly pigmented. Mandibles (Fig. 26) relatively broad, bifid, teeth of unequal height; slightly truncate; *mds1* very long, *mds2* distinctly short, piliform. Maxilla (Fig. 27) stipes with 1 *stps*, 2 *pfs* and 1 *mbs*; *stps* and *pfs1–2* very long, almost equal in length, *mbs* very short; mala with 14 bacilliform *dms* in two different lengths (1–4 long, blunt and 5–14 very long and blunt, with a tendency to be longer and less blunt); 5 relatively long *vms*, almost equal in length; *vms* distinctly shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with 1 very short *mxps* and two sensilla; length ratio of basal and distal palpomeres: 1:0.8; distal palpomere with one sensillum and a group of conical, apical sensorial papillae. Praelabium (Fig. 27) heart-shaped and distinctly elongated, with 1 relatively long *prms*; ligula with sinuate margin and 3 piliform micro *ligs*, unequal in length; premental sclerite well visible. Labial palpi with two palpomeres; length ratio of the basal and distal palpomeres: 1:0.6; distal palpomere with one one sensillum and short, apical sensorial papillae; basal palpomere with 1 ventral sensillum, and pigmented in the basal part, and the connection with the premental sclerite seems as next palpomere. Postlabium (Fig. 27) with 3 *pms*, *pms1* located anteriorly, remaining two pairs laterally; long, almost of equal length, *pms3* distinctly shorter than *pms1* and *pms2*; surface of postlabium densely covered by distinct asperities.

Thorax. Prothorax distinctly smaller than meso- and metathorax. Metathorax almost of equal length as abdominal segments I–IV. Spiracle bicameral. Prothorax (Fig. 29) with 10 *prns* unequal in length, of which 8 on distinctly pigmented dorsal sclerite that is subdivided medially into two triangular plates, next two *prns* placed below; 2 long *ps* located on pigmented sclerite, and 1 *eus*. Mesothorax (Fig. 29) with 1 long *prs*; 4 long to very long *pds*, *pds2* distinctly shorter than the remaining three setae (both on weakly pigmented sclerites); 1 very short *as*; 2 short *ss*; 1 *eps*; 1 *ps* (*eps* and *ps* on weakly pigmented sclerites) and 1 *eus*. Chaetotaxy of metathorax (Fig. 29) identical to that of mesothorax. Each pedal area of the thoracic segments well separated and pigmented, with 7 long *pda*, of which 4–6 on distinctly pigmented area, unequal in length.

Abdomen. Abdominal segments I–V of almost equal length and subsequent abdominal segments decreasing gradually to the terminal parts of the body. Abdominal



Figures 23–24. *Lixus neglectus*mature larva. **23** Labrum and clypeus **24** Epipharynx. Scale bar: 0.5 mm.



Figures 25–26. *Lixus neglectus* mature larva head. **25** Antenna **26** Right mandible. Scale bars: 0.1 mm (**25**) and 0.5 mm (**26**).

segment X reduced to four anal lobes of unequal size, the dorsal being distinctly the largest, the lateral pair equal in size, and the ventral lobe very small. Anus located terminally. Spiracles bicameral, the eight abdominal spiracles located laterally, close to the anterior margin of abdominal segments I–VIII. Abdominal segments I–VII (Figs 30–31) with 1 *prs*; 5 relatively short *pds*, *pds2* and *pds4* on abdominal segment VII less than half of length of the three remaining setae which are twice as long as *pds* on the previous six abdominal segments; 2 *ss* of unequal length, *ss1* very short, *ss2* as long as *pds5*; 2 *eps* of unequal length; 2 relatively short *ps* of equal length; 1 long *lsts* (*eps*, *ps* and *lsts* on weakly pigmented sclerites) and 2 relatively long *eus*. Abdominal segment VIII (Fig. 31) with 1 relatively long *prs*; 2 long to very long *pds, pds1–2* and *pds4* lacking; 2 *ss* of unequal length, *ss1* very short, *ss2* as long as *prs*; 2 *eps* of unequal length, *eps1* relatively long, *eps2* long to very long; 2 relatively short *ps* of equal length;

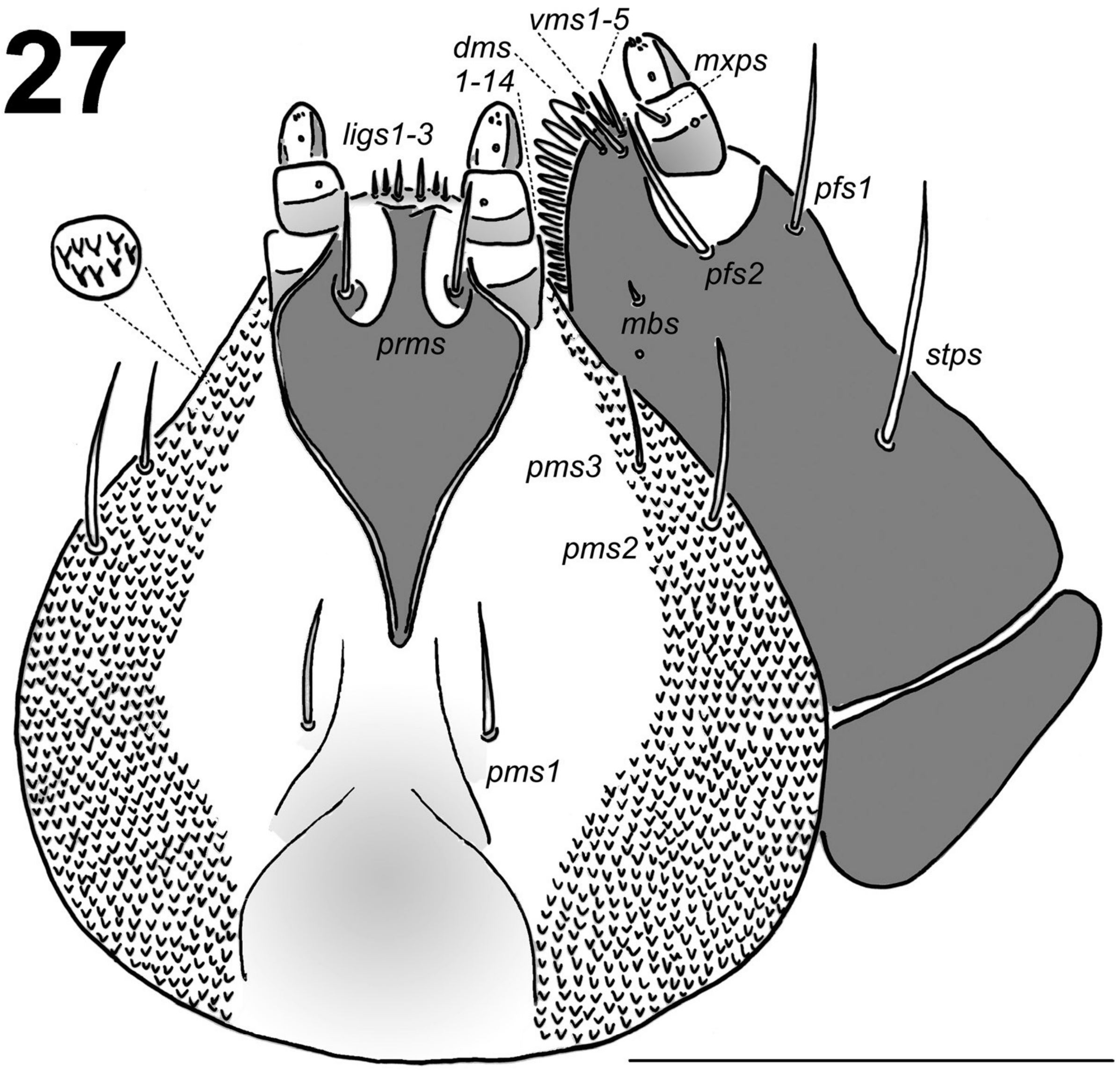


Figure 27. *Lixus neglectus* mature larva head, maxillo-labial complex, ventral view. Scale bar: 0.5 mm.

1 relatively long *lsts* (*eps*, *ps* and *lsts* on weakly pigmented sclerites) and 2 relatively long *eus*. Abdominal segment IX (Fig. 31) with 3 *ds* (*ds1–2* long, *ds3* short); 2 very short *ps* and 2 very short to micro *sts*. Abdominal segment X (Fig. 31) with 2 microsetae (*ts*), on each lateral lobe.

Description of pupa. *Measurements* (in mm). Body length: 9.4–12.7 (♂ 9.4–12.7; ♀ 10.0) and the widest part of the body, commonly between the apex of the meso- or metafemora: 2.8–3.8.

Colouration. Body yellow (Fig. 39).

Morphology (Figs 32–34, 39). Body stocky, elongated, white or yellowish. Cuticle smooth. Rostrum relatively long, approximately 3.1 to 3.5 times as long as wide and extending beyond the mesocoxae. Antennae relatively long and stout. Pronotum from 1.2 to 1.3 times as wide as long. Mesonotum and metanotum of almost equal length. Abdominal segments I–V of almost equal length; abdominal segment VI semicircular and subsequent abdominal segments diminish gradually to the end of the body. Ab-

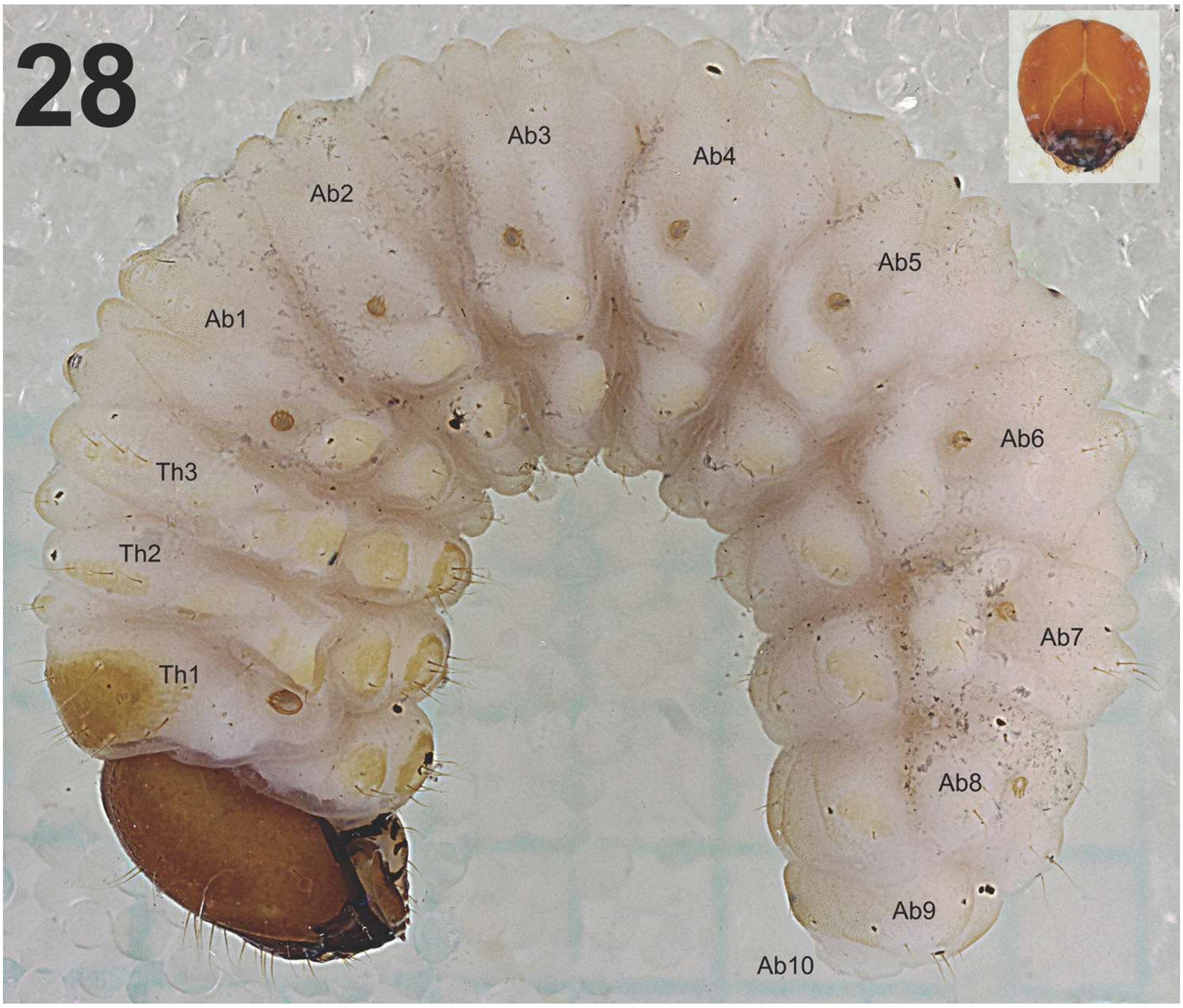
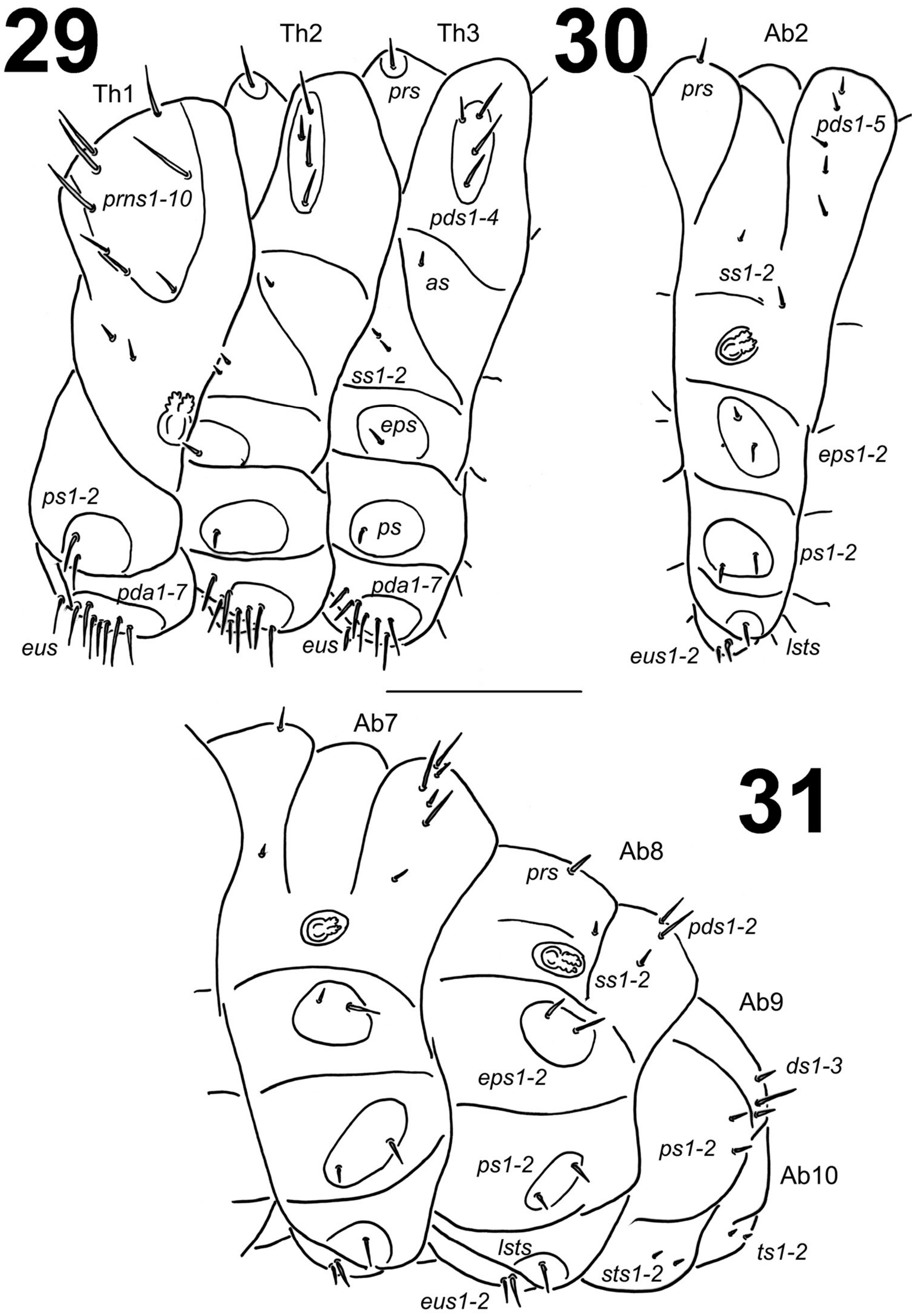


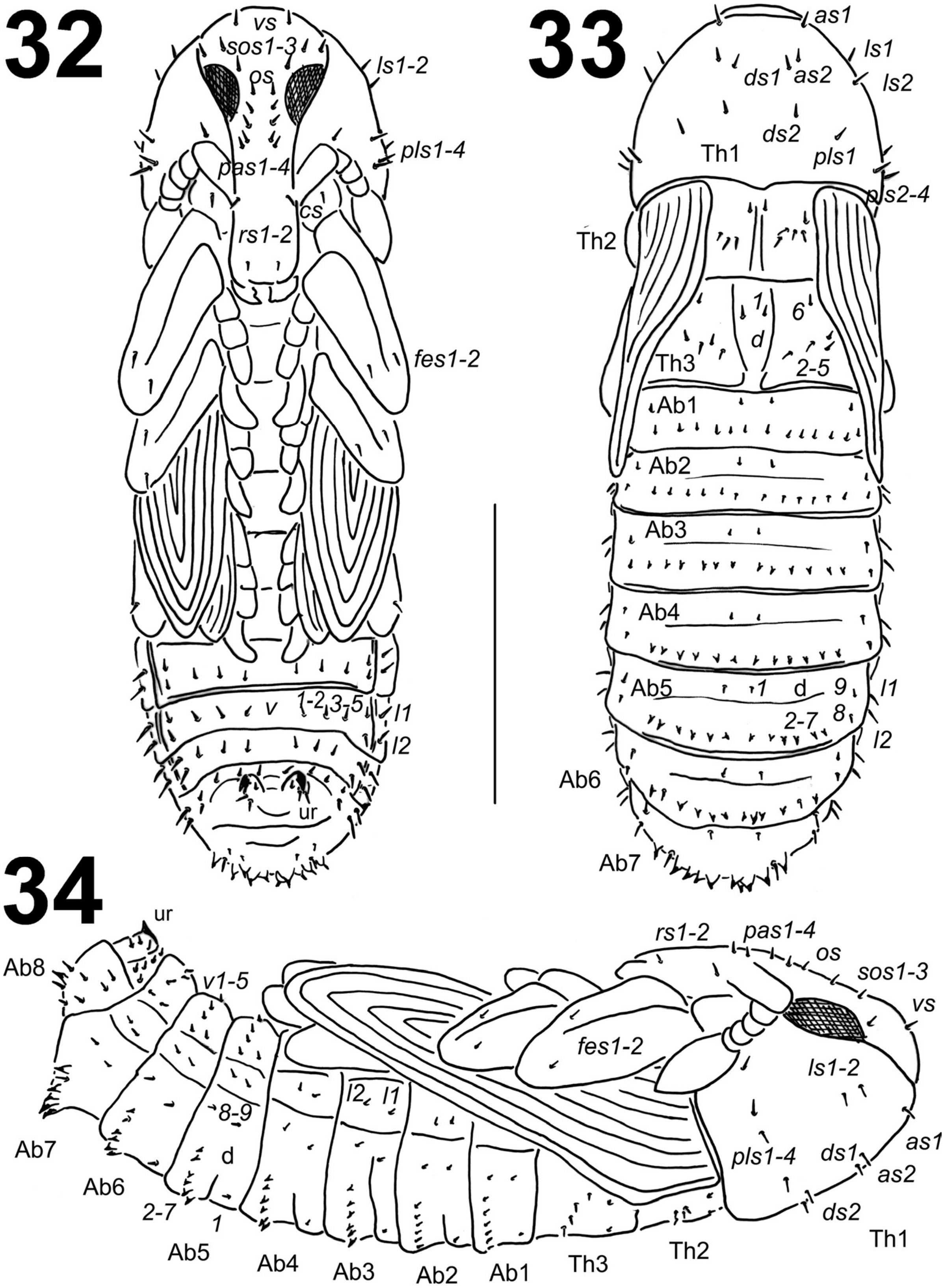
Figure 28. *Lixus neglectus* mature larva habitus, lateral view. Scale bar: 3 mm.

dominal segments VII–IX distinctly smaller than other abdominal segments. Gonotheca (abdominal segment IX) in females (1 specimen) bilobed.

Chaetotaxy (Figs 32–34). Setae relatively short, unequal in length, light yellow or orange, some setae on abdominal segments III–VIII distinctly stronger and located on protuberances. Setae well visible. Head capsule includes 1 *vs*, 3 *sos*, 1 *os* and 4 *pas*. Rostrum with 2 *rs*, *rs1* placed below antenna, *rs2* on the anterior margin. Setae on head capsule and rostrum straight, both *rs* and all *pas* distinctly shorter than the remaining setae on head, thoracic and abdominal segments. Pronotum with 2 *as*, 2 *ds*, 2 *ls* and 4 *pls*, and 1 short seta on ventral side (probably *pls5*). Dorsal parts of mesothorax with 1 seta located posteromedially, 1 seta located posterolaterally and 4 setae located along the anterior margin. Chaetotaxy of metathorax identical to that of mesothorax. Coxa with 1 very short *cs*. Each femoral apex with 2 *fes*. Dorsal parts of abdominal segments I–VIII each with 2 pairs of setae located posteriorly (*d1*, *d9*) and 7 pairs (*d2–8*) located along the anterior margins. Setae *d2–7* (on abdominal segments III–V) and setae *d2–5* and *d7* (on abdominal segments VI–VII) short, thorn-like, located on protuberances. Protuberances on abdominal segment VII distinctly prolongated. Remaining setae relatively short, hair-like. Abdominal segments I–VII with groups of 2 lateral setae and 5 pairs of



Figures 29–31. *Lixus neglectus* mature larva habitus. **29** Lateral view of thoracic segments **30** Lateral view of abdominal segment II. **31** Lateral view of abdominal segments VII–X. Scale bar: 1 mm.



Figures 32–34. *Lixus neglectus* pupa habitus. **32** Ventral view **33** Dorsal view **34** Lateral view. Scale bar: 3 mm.

Figures 35–42. Habitats, adults, immature stages, host plants and life cycle of *Lixus neglectus*. **35** Adult **36** Adult hiding in host plant rosette **37** Ovipositional mark and larva feeding marks (frass) **38** Mature larva **39** Pupa **40** Fresh, not fully coloured adult **41** Habitat in Czech Republic with host plant, *Rumex thyrsiflorus* **42** Detail of host plant rosette.

ventral setae. Dorsal part of abdominal segment VIII with 1 seta located posteriorly (*d9*) and 5 pairs (*d3–7*) located along its anterior margin; *d7* thorn-like, located on protuberances; remaining setae short. Abdominal segment VIII with groups of 2 lateral setae and 5 short ventral setae. Abdominal segment IX with 2 pairs of ventral microsetae and 1 pair of short, thin setae. Urogomphi distinctly elongated, hooked, triangular.

Biology and ecology

Habitats. Adults live in dry grasslands and meadows with sandy substrates (wind-blown river sand) (Fig. 41).The meadows are often managed for hay production. Numerous specimens were also found on grassy embankments along roads.

Adult behaviour. Adult beetles (Fig. 35) exhibit diurnal as well as nocturnal activity. During sunny days, they spend almost all of their time hiding among the leaves of the host plant near the ground (Figs 36, 42) and rarely climb to the higher parts of the plant.

Host plants. Adults and their immature stages were observed exclusively on dock, *Rumex thyrsiflorus* Fingerh. (Polygonaceae) (Figs 41–42), in all of our localities. In the past, this species was only recorded from garden sorrel (*Rumex acetosa*) L. (e.g., Fremuth 1983).

Life cycle. *Lixus neglectus* is an univoltine species. Adults feed on leaves, and larvae are stem and root borers (Fig. 38). Females of *L. neglectus* bite the lower part of the stem and lay one egg in the hole (Fig. 37). Larvae feeding in the root produce orange feeding frass (Fig. 37), which is thrown out of the host plant, and its presence is a reliable indication that the plant root is occupied by a larva. One plant is most likely occupied by only one larva. Mature larvae were found from July to August. Pupation takes place in the root neck (Fig. 39), and freshly hatched individuals can be found (inside plants) beginning in the middle of August (Fig. 40). Adults do not hibernate in the host plants, so hibernation most likely occurs in the leaf litter, among dry plant debris or in the topsoil.

Rearing of the larvae. For laboratory breeding, 15 mature larvae were collected on July 13th, 2014, but only two of them pupated in our laboratory conditions. The remaining larvae died primarily due to drying of the host plants. Both of the adults hatched on July 30th.

Discussion

Comparison with larvae of other *Lixus* species. To date, larvae of 21 *Lixus* and two *Hypolixus* species have been described (Scherf 1964; Lee and Morimoto 1988; May 1994; Nikulina 2001, 2007; Zotov 2009a, b; Nikulina and Gültekin 2011; Gosik and Wanat 2014; Skuhrovec and Volovnik 2015), but a detailed description of the pupae is known for only 8 *Lixus* species (Scherf 1964; May 1994; Zotov 2009a, b; Gosik and Wanat 2014; Skuhrovec and Volovnik 2015).

The precise general description of the larvae of the genus *Lixus*, which can be summarized by 19 character sets, has been presented by May (1994) and more in detail by Nikulina (2001) (for details, see Nikulina 2001 and Gosik and Wanat 2014). The larvae of *L. bituberculatus* and *L. neglectus* possess all of these characters, with only a few exceptions that partly result from differences in terminology (for details, see Gosik and Wanat 2014). Nikulina (2001) also published the only comprehensive and known larval key for this genus. The larva of *L. bituberculatus* has the closest affinity to the larva of *L. kiritshenkoi* Ter-Minasian, 1985 (abdominal segment X with two setae on the ventral side, lateral sides without setae, and dorsal side with less than three setae, see Table 1). The main differences are as follows (see Table 1): prothoracic tergite with 10 *prn* (vs. 13 *prn*); maxilla stipes with short 1 *mbs* (vs. without *mbs*, which could also be easily overlooked because it is very often minute); prodorsum on abdominal segment IX with only 4 *ds* (vs. 3 *ds*), and 3 short *ps* (vs. 1 *ps*). The larva of *L. bituberculatus* is the first of the immature stages described for the subgenus *Ortholixus*, but the larva of *L. neglectus* does not fit the description of any larva in the key (Nikulina 2001) because the first step provides no option for abdominal segment X to be without setae on the dorsal side (see Fig. 31). An interesting characteristic of *L. neglectus* is the presence of more pigmented sclerites on the larval body (see Figs 28–31, Table 1), which is similar to the description of *L. filiformis* (Nikulina and Gültekin 2011). In the subgenus *Dilixellus*, to which *L. neglectus* belongs, the larvae of four species have already been described: *L. bardanae* (Fabricius, 1787) (in Scherf 1964); *L. desbrochersi* Hoffmann, 1957 (in Lee and Morimoto 1988 as *L. impressiventris* Desbrochers des Loges, 1904); *L. probus* Faust, 1887 (in Nikulina 2001); and *L. punctiventris* Boheman, 1835 (in Gosik and Wanat 2014) (see Table 1). The creation of a precise key and a detailed subgeneric study of the genus *Lixus* is currently limited due to the lack of knowledge of the immature stages (see Table 1). The main problem is that we are unable to divide with certainty the morphological characteristics of this group into (i) characteristics that are useful for phylogenetics and (ii) characteristics that are useful only for species identification. Once this categorization is complete, it will be possible to apply it for future cladistics analysis, which are planned for the near future. All these morphological data should be compared and correlated with known biological data of the host plant families for the different groups of *Lixus* and also related genera, because some subgenera of *Lixus* are probably composed of different probably unrelated groups developing on quite different families of plants (Skuhrovec, Gosik, Stejskal, Trnka, Volovnik, Gültekin, unpublished data).

May (1993) considered the increased number of *pds* on the meso- and metathorax and abdominal segments I–VII and the increased number of setae on the epipharyngeal lining (*als*) (i.e., higher than the most frequent number of setae in weevils) as diagnostic of the mature larva of the Lixinae subfamily, and the descriptions of mature larvae from the tribe Lixini (*Larinus* species: Zotov 2009a, 2010; Gosik and Skuhrovec 2011; *Lixus* species: Scherf 1964; Lee and Morimoto 1988; May 1994; Nikulina 2001, 2007; Zotov 2009a, b; Nikulina and Gültekin 2011; Gosik and Wanat 2014; Skuhrovec and Volovnik 2015; *Rhinocyllus conicus*: May 1994) fit this diagnosis, as do all known

Table 1. Differential diagnosis of mature larvae of both described species and the most similar or relative species.

	***L.* (*Dilixellus*) *bardanae* (Fabricius, 1787)**	***L.* (*Dilixellus*) *desbrochersi* Hoffmann, 1957**	***L.* (*Dilixellus*) *neglectus* Fremuth, 1983**	***L.* (*Dilixellus*) *probus* Faust, 1886**	***L.* (*Dilixellus*) *punctiventris* Boheman, 1835**	***L.* (*Epimeces*) *filiformis* (Fabricius, 1781)**	***L.* (*Eulixus*) *kiritshenkoi* Ter-Minasian, 1985**	***L.* (*Ortholixus*) *bituberculatus* Smreczyński, 1968**
Endocarina	unknown	present, length unknown	more than the half length of frons	short	more than the half length of frons	more than the half length of frons	more than the half length of frons	more than the half length of frons
Number of *des*	unknown	4	5	5	5	5	5	5
Number of *fs*	unknown	5	5	3	5	5	5	5
Number of *les*	unknown	2	2	unknown	3	1	unknown	2
Number of *ves*	unknown	2	2	unknown	2	unknown	unknown	2
Position of *lrms1-3*	in a triangle	only 2 setae	in a triangle	in a triangle	in a triangle	in a triangle	in a line	in a triangle
Number of *als*	unknown	6	4	4	5	3	3 (or 4)	4
Number of *ligs*	2	2	3	3	2	2	1	3
Number of *mbs*	1	unknown	1	1	2 ?	1	0	1
More pigmented sclerites on larval body, not only on pronotum	unknown	absent	present	absent	absent	present	absent	absent
Number of *prns*	unknown	8	10	10	10	10	13	10
Number of *pds* on Abd. seg I- VII	unknown	5	5	unknown	6	6	unknown	6
Number of *ds* on Abd. seg IX	unknown	unknown	3	unknown	6	2	3	4
Number of *ps* on Abd. seg IX	unknown	unknown	2	unknown	3	2	1	3
Number of *ts* on dorsal lobe on Abd. Seg. X	unknown	unknown	0	7 *	0	0	2 *	0
Number of *ts* on ventral lobe on Abd. Seg. X	unknown	unknown	0	2 *	0	0	2 *	0
Number of *ts* on lateral lobe on Abd. Seg. X	unknown	unknown	2	0 *	3	3 (dorsoventral)	0 *	3

* Nikulina (2001) listed number of *ts* on sides, not on lobes.

species from the tribe Cleonini (Stejskal et al. 2014, Trnka et al. 2015). Currently, the comparison of both tribes, including key and detailed generic studies, is impossible due to our limited knowledge of the immature stages. A categorization of the morphological characteristics of Cleonini and a comparison of both tribes is planned following a detailed study of the genus *Lixus* (see the previous paragraph). The presence of 5 *vms* in the maxillary mala observed in all Lixinae and also most curculionids could be helpful as differential character from the root feeder larvae in Entiminae which have only 4 *vms* (Marvaldi 1998a, b).

Biology and ecology

The biology and development of these two *Lixus* species are very similar as both species are stem and root (crown) borers. In the genus *Lixus*, root borers, such as *L.* (*Ortholixus*) *angustus* (Herbst, 1795); *L.* (*Compsolixus*) *ochraceus* Boheman, 1842; *L.* (*Dilixellus*) *punctirostris* Boheman, 1842; *L.* (*Dilixellus*) *punctiventris* Boheman, 1835; and *L.* (*Ortholixus*) *vilis* (Rossi, 1790) (Dieckmann 1983) have not been identified as frequent. The prevailing nocturnal activity of the adults and the species hidden life habits are probably the main reasons why these species have not been found elsewhere, and it is very difficult to confirm them at a locality. The majority of the Central European Lixinae species require a specific habitat disturbance regime that results in sparse vegetation cover (Stejskal and Trnka 2013, 2014). In both species, it seems that adults and the immature stages prefer places with pasture vegetation or meadows that are managed for hay production.

This is the first report of *L. bituberculatus* from Romania, and it probably has a larger area of distribution including Bulgaria, Hungary, Romania and Slovakia (Gültekin and Fremuth 2013; Stejskal and Trnka 2014). Based on our observations *L. bituberculatus* appears to be oligophagous on Asteraceae; all of our specimens were only found on *Cichorium intybus*, which originates from the Mediterranean but has been introduced to North America, southern Africa and New Zealand (Dvořáková 2005). It seems that this weevil is not specialized on only one plant species because it was found on *Crepis* sp. as well as *Picris* sp. in Slovakia, so its suitability as a candidate for the biological control of *C. intybus* is questionable. Only a host plant choice test can help us to determine all of the host plant species and the preferences of this species.

Lixus neglectus has been found exclusively on *Rumex thyrsiflorus*, but the distribution of its host plant covers the majority of Europe (Kubát 1990). However, this beetle is only known from a very small and specific area. The sorrel *R. thyrsiflorus* is considered to be a naturalized neophyte in Central Europe (Danihelka et al. 2012), but its origin remains unknown (Kubát 1990). This *Lixus* species seems to be monophagous, and its only host plant, *R. thyrsiflorus*, is not considered to be harmful and has not yet been introduced to other continents or countries. Therefore, it is not necessary to utilize this weevil to regulate its host plant despite its suitability as a biological control agent, but this must be validated by future studies. The distribution of *L. neglectus* has to be confirmed, especially across the wider area of distribution of *R. thyrsiflorus*. This weevil can be easily overlooked due to its cryptic way of life, but the easiest way to confirm its presence in the field is to search for frass at the root crown of the host plant. This frass is a very unique behaviour within the genus *Lixus*. To date, this weevil has only been recorded from *R. acetosa* (e.g., Fremuth 1983; Koch 1992; Böhme 2001), which is likely due to misidentification as occurred in our case (Trnka and Stejskal 2014). It is possible that *L. neglectus* historically lived on *R. acetosa* but has recently come to occupy a new available ecological niche on expanding *R. thyrsiflorus* (from ca 15th century). However, because each of the host plants prefer different habitats, this explanation is quite unlikely. The common sorrel, *R. acetosa*, prefers wet habitats, whereas the compact dock, *R. thyrsiflorus*, prefers dry ones (Kubát 1990). Both sorrels are also known as host plants for some other weevils, but there does not seem to be any competition among the species. Development on *R. acetosa* is also known in one other *Lixus* species, *L. bardanae* (Fabricius, 1787), but this weevil is a typical stem borer. Thus there is no competition between it and *L. neglectus* (Dieckmann 1983). A similar situation occurs in the development of some Apionids (e.g., *Apion cruentatum* Walton, 1844 or *Perapion oblongum* (Gyllenhal, 1839)), whose larvae also feed only in the stems (Dieckmann 1977). Furthermore, both plants host the oligophagous weevil species *Marmaropus besseri* Gyllenhal, 1837 (Dieckmann 1972) in their stems and roots, and this species has been recently expanding along with *R. thyrsiflorus* (Rheinheimer and Hassler 2010). Both of these weevils belong to the same guild (stem and root (crown) borers), so there could be some competition. However, this requires more information on the timing of their development as well as some of the other abiotic and/or biotic effects inside the plant. Thus, the complete switch in host plant by *L. neglectus* seems unlikely, mainly due to its rarity despite the recent expansion of its host plant. It is more probable that its preference for *R. thyrsiflorus* as a host plant is recent, but this could only be resolved through a host plant choice test.

Knowledge of the immature stages and life histories of insects can help protect endangered species (including the species presented here) more effectively. The detailed descriptions of the larva and pupa and their comparison with known descriptions reported here demonstrates the possibility of identifying species in their immature stages. Future detailed biological and morphological studies can yield unique information on the factors determining host specificity in this insect group and will provide useful background information for planning efficient biocontrol of invasive plant species. The issue of using some insects as biological control agents is a key topic in both the basic and applied research on invasive plants. Our results will significantly contribute to basic research but will also have practical implications for conservation biology and/or biological control.

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RESEARCH ARTICLE

Velcro-Like System Used to Fix a Protective Faecal Shield on Weevil Larvae

Jiří Skuhrovec[1]*, **Robert Stejskal**[2], **Filip Trnka**[3], **Andrea di Giulio**[4]

1 Group Function of Invertebrate and Plant Biodiversity in Agro-Ecosystems, Crop Research Institute, Praha 6 – Ruzyně, Czech Republic, **2** Administration of Podyji National Park, Znojmo, Czech Republic, **3** Department of Ecology & Environmental Sciences, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic, **4** Department of Science, University Roma Tre, Rome, Italy

* jirislavskuhrovec@gmail.com

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Abstract

The last instar larva and pupa of *Eucoeliodes mirabilis* (A. Villa & G. B. Villa, 1835) (Curculionidae: Ceutorhynchini) are described using drawings and SEM images and are compared and keyed with already described larvae of 58 other ceutorhynchinae taxa. The larval body has an effective combination of morphological adaptations that assist a unique biological defensive strategy. All larval stages of *E. mirabilis* feed ectophytically on leaves of *Euonymus europaeus* L. (Celastraceae), and the larval body is covered with a thick faecal shield. The fixation of this protective shield on the larval back is performed by a peculiar dorsal microsculpture composed of a dense carpet of microtrichia on the thorax and abdomen, which serves effectively as a velcro system. Because of this strategy, macrosetae on the larval and pupal body of *E. mirabilis* are completely reduced. Larvae of *E. mirabilis* also have distinct morphological adaptations for protecting the spiracles against intrusion of faeces and avoiding occlusion of the tracheal system: a) microtrichia around spiracles are slightly shorter, distinctly stronger and are arranged with high-density and in clusters and b) spiracles are protected by an external safety valve. This strategy of *E. mirabilis* larvae is unique, although somewhat similar to that of Criocerinae and Blepharida-group leave beetles (Galerucinae) (both Coleoptera: Chrysomelidae), but with distinctly different morphological adaptations.

Introduction

The endless adaptations and counter-adaptations among three trophic levels (plants, herbivores and their enemies) are responsible for the amazing variety of defensive strategies recorded for both plants and their herbivorous insects [1]. To avoid the attacks of predators and parasitoids, herbivorous insects have developed many types of defensive strategies that vary from avoiding detection using visual camouflage to deceiving enemies by mimicking unpalatable species [2]. One of the most astonishing defensive strategies may be the faecal ecology of insects, which has received some attention [3]. Immature stages of five clades of leaf beetles, Cassidinae, Criocerinae, Cryptocephalinae, Lamprosomatinae and Galerucinae, use faeces as a material for building domiciles and protective shields/coats against their enemies [4–20]. The shields provide a mechanical defence and may also include chemicals that originated from the host plant or derivatives of these compounds produced by the larvae [21–23].

Each of these clades of leaf beetles shows slight differences in the use of faeces. The strategy of using faeces as a shield is most recognized for the larvae of tortoise beetles, Cassidinae [15]. Eisner and his colleagues [4] first reported that the faecal shields of tortoise beetle larvae are the primary defensive strategy against predators. Some larvae of Cassidinae have special superanal processes to carry the defensive shields, which are composed of shed larval skins (exuviae) and faeces that are often retained after pupation [15]. These larvae can move this defensive shield to the side, in contrast to the remaining four groups (Criocerinae, Cryptocephalinae, Lamprosomatinae and Galerucinae), which have faeces only on the dorsum and cannot move the defensive shield from side to side. Larvae in the clade Lamprosomatinae and Cryptocephalinae, sister taxa referred to as Camptosomata, are defined by a bell-shaped case from faeces and plant material as portable enclosures, but the head and legs are not protected during walking [16, 17]. Larval chaetotaxy of both these groups is reduced [16, 17]. In Galerucinae, only larvae of four genera, *Blepharida* Chevrolat, *Diamphidia* Gerstaecker, *Podontia* Dalman, and *Polyclada* Chevrolat, use faecal coats [13, 24]. All larval instars partially or completely cover the terga with faeces, resembling semirigid pellets or a wet mass. Larvae of Criocerinae have the anus oriented vertically, and then digestive wastes (faeces) accumulate on the dorsum to partially or entirely cover the larvae [18]. A faecal shield acts primarily as a physical and chemical barrier with deterrent metabolites that include fatty acids (e.g., octadecanoic acid and phytol). Larvae continuously form this protective shield after each moulting, and the dorsal vesture consists of short, relatively sparse setae [8, 18, 25].

During biological investigations on the weevil *Euceliodes mirabilis* (A. & G. B. Villa, 1835), we observed some protective behaviours of the larvae that included an ectophytic habitat and the use of a faecal shield. To date, this peculiar defensive strategy is known only in the family Chrysomelidae. *Eucoeliodes* Smreczyński, 1974 is a rare, monotypic genus [26] formerly regarded as a subgenus within *Coeliodes* Schoenherr, 1837 [27, 28], and now considered as genus related to the genus *Coeliodes* and other relative genera as e.g. *Neocoeliodes* Colonnelli, 1984 [29]. The distribution of *E. mirabilis* includes Central Europe (Austria, Czech Republic, Hungary, Slovakia and Switzerland) and a part of southern Europe (Croatia, Italy and Slovenia) [26]. The information on the biology of this weevil is very poor, although reports are available that the weevil is collected on European spindle (*Euonymus europaeus* L., Celastraceae) [27–30] and alder buckthorn (*Frangula alnus* Mill., Rhamnaceae) [31]. Numerous populations of *E. mirabilis* were found in two localities in southern Moravia (Czech Republic) at the northernmost edge of the range of the species. In this paper, we present new biological and morphological data on the larval development and behaviour of the weevil *Eucoeliodes mirabilis*.

Materials and Methods

Insect collection and laboratory breeding

The material used to describe immature stages of *Eucoeliodes mirabilis* was collected, and field observations were conducted in two study areas: 1) Czech Republic, Moravia mer., National Park Podyjí, Znojmo env., 250 m a.s.l., visit dates: 19.v.2010, 2 ex.; 29.iv.2012, ca 30 ex. (adults); 10.v.2012, ca 20 ex. (only observed, adults and larvae), leg. Robert Stejskal (RS); 19.v.2012, more than 100 larvae (only observed on three bushes), leg. RS; 30.v.2014, more than 100 larvae (only observed on three bushes)–ca 10 ex. collected for rearing, leg. RS; 7.vi.2014, 2 adults and ca 20 larvae (only observed on three bushes), leg. RS and 2) Czech Republic, Moravia centr., Boskovice env., 350 m a. s. l., visit dates: 28.vii.2012 (old feeding marks on leaves), 23.v.2015,

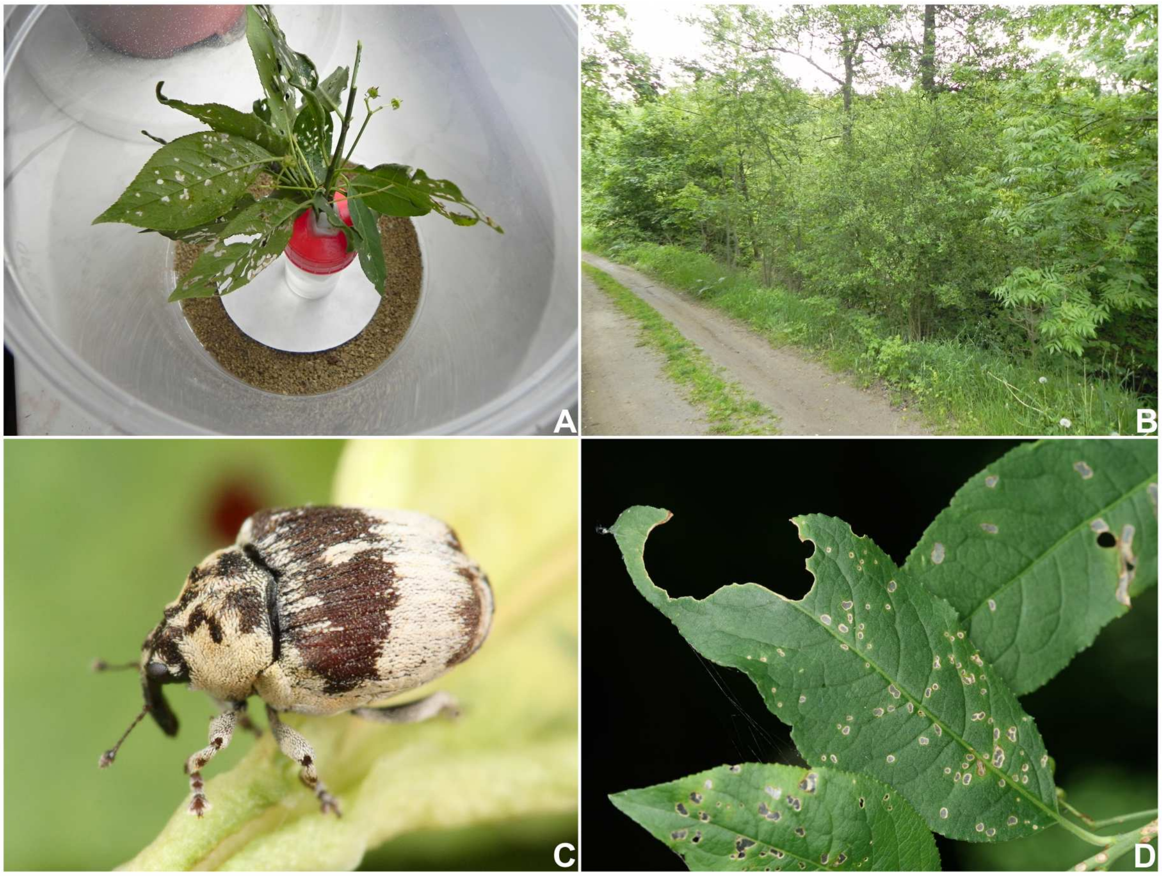

Fig 1. *Eucoeliodes mirabilis*: adult, habitat and host plant. (A) Larvae rearing in a plastic container with a few branches of *Euonymus europaeus*. (B) Habitat in the Boskovice (Czech Republic). (C) Adult. (D) Feeding marks of adults. Photos A and B by R. Stejskal, and C and D by F. Trnka.

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ca 30 adults (7 collected) and ca 100 small larvae (observed on ca 10 bushes)–ca 20 larvae collected for rearing, leg. RS. Descriptions of immature stages were performed on ten larvae and five pupae.

Laboratory observations were conducted in Znojmo, Czech Republic. Adults and larvae were placed in a covered small plastic container with a few branches of *Euonymus europaeus* (Fig 1A). Fine sand obtained in a pet shop was placed at the bottom of the container. The container containing the host plants and larvae was placed near the window at room temperature and slightly moistened approximately once every 3–5 days.

To define the concepts of endo/ectophyty and endo/ectophagy, we followed Oberprieler et al. [32]: endophyty refers to living inside plant tissues, whereas endophagy denotes feeding inside any substrate (e.g., soil, water). Similarly, ectophagous species feed and live on the surface of a host, and when the host is a plant, the species are ectophytic.

Morphological descriptions

Part of the larval and pupal material was preserved in Pampel fixation liquid (4 parts glacial acetic acid, 6 parts 4% formaldehyde, 15 parts 95% ethanol and 30 parts distilled water) and used for the morphological descriptions. These specimens are now deposited in the Group Function of Invertebrate and Plant Biodiversity in Agro-Ecosystems of the Crop Research

Institute (Prague, Czech Republic). The collectors identified the plants. To prepare the slides, we followed May [33]: a larva was decapitated, and the head was cleared in a 10% potassium hydroxide (KOH) solution and then rinsed in distilled water. After clearing, the mouthparts were separated from the head capsule, and the head capsule and all mouthparts were mounted on permanent microscope slides in Euparal. All other body parts were mounted on temporary microscope slides in 10% glycerine.

The observations and measurements were conducted using a light microscope with calibrated oculars (Olympus BX 40 and Nikon Eclipse 80i). The following characteristics were measured for each larva: head width, length of the body (larvae fixed in a C-shape were measured in segments), and width of the body in the widest place (i.e., metathorax or abdominal segments I–IV). For the pupa, the length and the width at the widest place were measured. The thorax and abdomen were not sclerotised, and it is unlikely that the fixation process altered the proportions of the weevils; measurements of these parts are provided for comparison purposes only.

Drawings were created with a drawing tube on a light microscope and edited using programs as Adobe Photoshop 10, Corel Photo-Paint 11, and GIMP 2. The thoracic spiracle is located on the prothorax near the boundary of the prothorax and mesothorax, as shown in the drawing (see Morphology of mature larva), but this spiracle is of mesothoracic origin [34, 35]. Drawings of body illustrate the thoracic and abdominal spiracles; the numbers of setae of the bilateral structures are given for one side.

We used the terms and abbreviations for the setae of the mature larva and pupa found in Scherf [36], May [33, 37] and Marvaldi [38, 39].

Scanning electron microscopy

To clean the specimens, larvae preserved in EtOH 70% were gradually re-hydrated to distilled water, maintained overnight in oven at 45°C in a detergent-water solution (10% Svelto® dishwashing liquid in distilled water), cleaned manually with a thin brush, and rinsed 3 times in distilled water. Then, the specimens were dehydrated by passing through a graded ethanol series, critical point-dried in a Balzer Union CPD 030 unit, gold coated in an Emitech K550 unit, and finally examined using the field emission SEM column of a Dualbeam (FIB/SEM) Helios Nanolab (FEI Company, Eindhoven, The Netherlands) at the L.I.M.E. (Roma Tre University, Rome, Italy), with secondary electrons (SE) and an operating voltage of 5 kV.

Results

Eucoeliodes (s.str.) *mirabilis* (A. & G. B. Villa, 1835)

Biological notes. **Habitat**: In both study areas (Znojmo env., Boskovice env.), the weevil occurred in mixed broadleaved forests near watercourses. Tree/shrub layer: *Fraxinus excelsior* L. (Oleaceae), *Alnus glutinosa* (L.) Gaertn. (Betulaceae), *Robinia pseudoacacia* L. (Fabaceae), *Acer campestre* L. (Sapindaceae), *Ulmus laevis* Pall. (Ulmaceae), *Euonymus europaeus* L. (Celastraceae), and *Sambucus nigra* L. (Adoxaceae). Herb layer: *Aegopodium podagraria* L. (Apiaceae), *Lamium maculatum* L. (Lamiaceae), *Galium aparine* L. (Rubiaceae), *Chelidonium majus* L. (Papaveraceae), *Impatiens parviflora* DC. (Balsaminaceae), and *Urtica dioica* L. (Urticaceae). The weevil preferred partly shadowed shrubs growing on forest edges (e.g., along roads and meadow edges, among others) (Fig 1B). Of note, although *Euonymus europaeus* was widely distributed in the study areas, the weevil occurred locally.

Adult activity: Adults (Fig 1C) appeared on the host plants at the end of April and immediately began to feed on leaves and twigs, where small holes were chewed (Fig 1D). Mating couples were observed from the end of April to the beginning of May. The last adults were

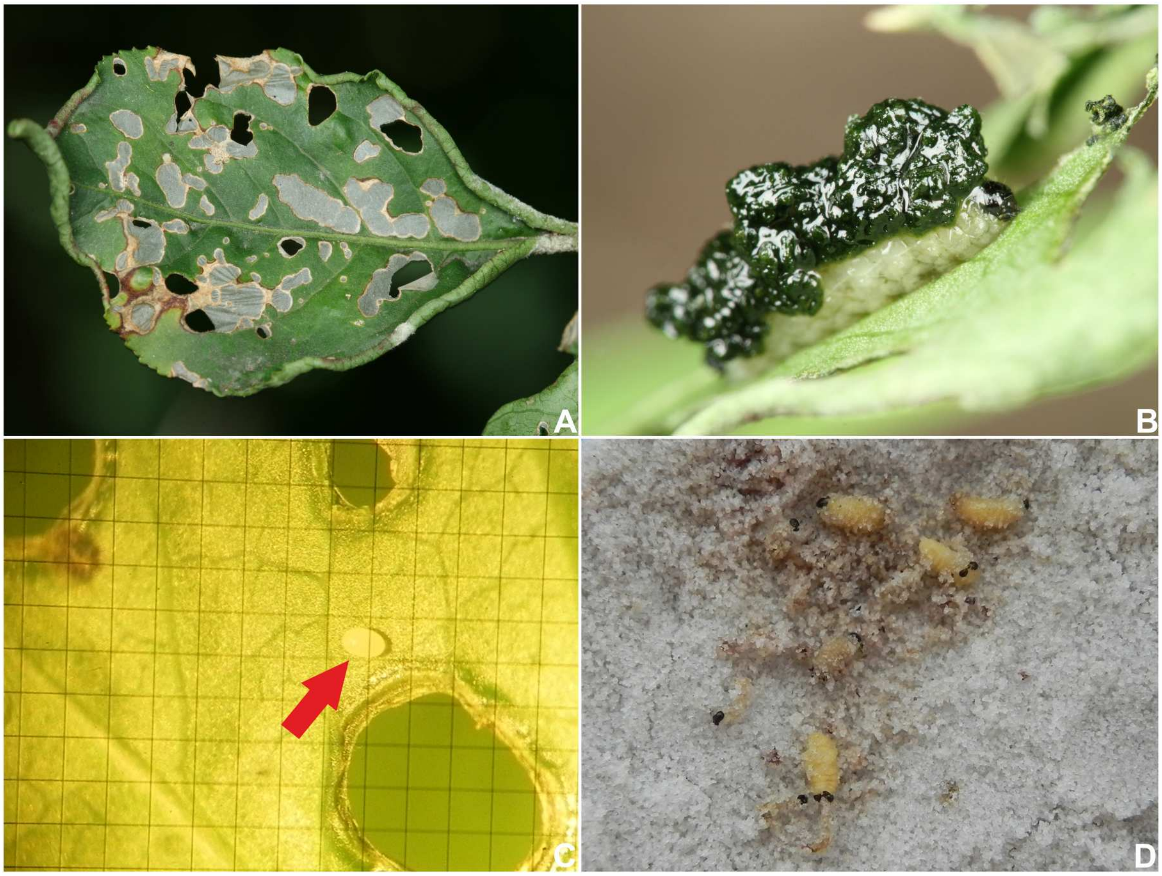

Fig 2. *Eucoeliodes mirabilis*: adult, host plant and larvae. (A) Feeding marks of larvae. (B) "Faecal shield" on larva. (C) An egg on a leaf of *Euonymus europaeus*. (D) Free pupae without any additional protection in the laboratory conditions. Photos A and B by F. Trnka; and C and D by R. Stejskal.

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observed to the beginning of June. In our database, we have records of adult *E. mirabilis* from other localities from the second half of June, which might indicate a new generation. No collecting data from summer and autumn months were available for the study areas or in other localities and published sources. The weevils may overwinter as adults.

Larval development: Larvae were observed from the 10th May to mid-June. The lower surface of a leaf was the site for larval feeding (Fig 2A). The larval body was covered with a "faecal shield"–viscid mucus and a dark substance reminiscent of excrements (Fig 2B). Young larvae resembled, at a glance, a small bird dropping and therefore could be easily overlooked. Depending on the age of the larva, feeding marks left patterns of different sizes and shapes on a leaf (Fig 2A and 2B). Initially, the feeding of young larvae is only superficial, and the epidermis remains largely untouched, whereas the feeding of older larvae leaves small holes or "windows" on the leaf (Fig 2A). A leaf can host up to 2 larvae. Feeding marks are very conspicuous; therefore, occupied host plants are easy to identify in the field, particularly at the end of May when the last larval instars feed intensively. We found no pupae in the field either on the host plants or in the litter on the ground.

Rearing experiments: Adults in captivity (ca 20 specimens) fed readily on host plants, mated, and laid only a total of 3 eggs, which dried out. Mating and oviposition were observed at the beginning of May 2012. The eggs were found on the lower surface of a leaf and were oval and lightly-colored, ca. 1.1 mm in length and ca. 0.7 mm in width (Fig 2C). The success in rearing the larvae, collected from the host plants in the field, was limited. Soon after placement on leaves in the rearing container, the larvae lost the protective shield, fed reluctantly, and moved around chaotically. Most specimens withered day by day and died by the end of the study. Pupation was observed in a few specimens (N = 4), and the larvae pupated on the surface of the sand that covered the bottom of the rearing dish. The pupae were free, without any additional protection, e.g., cocoon or earthen cell (Fig 2D). For larvae collected on the 23rd May, the pupation date was between the 3rd and 6th June.

Morphology of mature larva. **Measurements (in mm)**: Body length: 6.0–7.0 (mean: 6.5). The widest place in the body (abdominal segments II–V) measured up to 2.0. Head width: 0.6–0.7 (mean: 0.6).

Colouration: Black or dark brown head (Fig 2B). All thoracic and abdominal segments white (Fig 2B and 2D); only dorsum of pronotum with elongated, light brown stripe.

Head capsule: Head suboval, flattened laterally, endocarinal line absent (Figs 3A and 4A). Frontal sutures on head distinct, extended to antennae. Anterior and also posterior stemmata present, but feebly visible. *Des1* and *des2* located on upper part of the central part of epicranium, *des1* near the medial part of epicranium and *des2* near laterad of epicranium, *des3* located anteriorly near frontal suture, *des4* located in the central part of epicranium, and *des5* located anterolaterally; *des1–3* and *des5* relatively long, almost equal in length, *des4* very short to minute (Figs 3A and 4A). *Fs1* and *fs3* minute, placed medially, *fs2* absent, *fs4* relatively long, located anterolaterally, and *fs5* minute to very short, located laterally, close to the epistoma (Figs 3A and 4A). *Les* as long as *des1*; *ves1–2* very short (Fig 4B and 4D). Epicranial area with three setae (*pes1–3*) and 2 sensilla. Antennae located at the end of the frontal suture on each side, membranous and slightly convex basal article bearing conical, triangular sensorium, short; basal membranous article with 4 sensilla of different shape (Figs 3D, 4C and 4D). Clypeus (Figs 3E and 4C) approx. 2.5 times as wide as long, with 2 short *cls*, almost equal in length, located posterolaterally, and 1 sensillum located between *cls*; anterior margin concave.

Mouthparts: Labrum (Figs 3E and 4C) approximately 2.5–3 times as wide as long, with 3 pairs of piliform *lms* of different length; *lms3* very short, distinctly shorter than short *lms1* and *lms2*; *lms1* placed close to the margin with clypeus, *lms2* located anteromedially and *lms3* located anterolaterally; anterior margin double sinuate. Epipharynx (Fig 3F) with 3 blunt, slender, finger-like *als*, unequal in length; 2 very short, finger-like *ams*, *ams1* distinctly larger than *ams2*; 2 pairs of very short, blunt *mes*, unequal in length; labral rods (lr) elongated, sub-ellipse. Mandibles (Fig 3C) broad, trifid, tooth of unequal height; slightly truncate; both *mds* short, hairform, located in distinct alveoli. Maxilla (Fig 3B) stipes with 1 *stps*, 2 *pfs* and 1 *mbs*, *stps* and *pfs1–2* relatively long, almost equal in length, *mbs* very short; mala with 6 bacilliform *dms*; 5 short *vms*, distinctly different in length, 2 very short and 3 minute; *vms* distinctly shorter than *dms*. Maxillary palpi with two palpomeres; basal palpomere with 1 very short *mxps* and two sensilla; length ratio of basal and distal palpomeres: 1:0.9; distal palpomere with one sensillum and a group of conical, cuticular apical processes. Praelabium (Fig 3B) heart-shaped, with 1 short *prms*; ligula with sinuate margin and 1 piliform very short to minute *lig*; premental sclerite strongly sclerotized in ring-shaped with projections in the middle part (Fig 3B). Labial palpi with two palpomeres; length ratio of basal and distal palpomeres: 1:0.8; distal palpomere with one sensillum and short, cuticular apical processes; basal palpomere with 1 dorsal sensillum. Postlabium (Fig 3B) with 3 *pms*, *pms1* located anterolaterally, remaining two pairs more dorsolaterally; *pms1* and *pms3* very short to minute, *pms2* relatively long; surface of postlabium partly sclerotised.

Thorax and abdomen: Body elongate, slightly curved, rounded in cross section (Fig 5A–5C). Prothorax distinctly smaller than meso- and metathorax. Abdominal segments I–V of

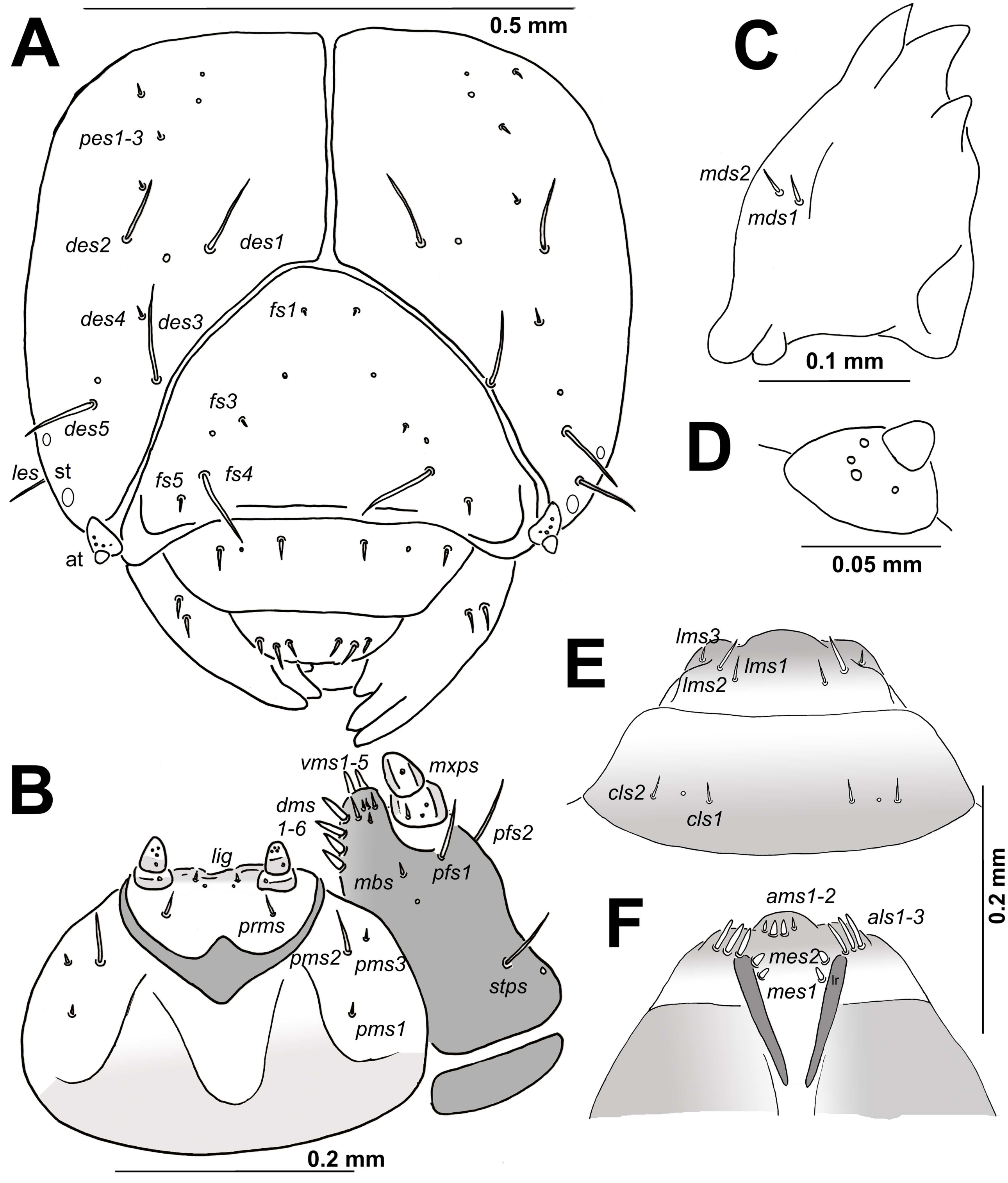

Fig 3. *Eucoeliodes mirabilis*: head and mouthparts of mature larva. (A) Anterior view of head. (B) Labium with left maxilla, ventral view. (C) Right mandible. (D) Antenna. (E) Labrum and clypeus. (F) Epipharynx. Abbreviations: *des*–dorsoepicranial seta(e), *fs*–frontal s., *les*–lateroepicranial s., *ves*–ventroepicranial s., at—antenna, st—stemmata, *cls*–clypeal s., *lms*–labral s., *ams*–anteromedial s., *als*–anterolateral s., lr—labral rods, *mds*–mandible dorsal s., *dms*–dorsal malae s., *vms*–ventral malae s., *mxps*–maxillary palps s., *pfs*–palpiferal s., *stps*–stipal s., *mbs*–mandible basiventral s., *prms*–prelabial s., *pms*–postlabial s., and *ligs*–ligular s.

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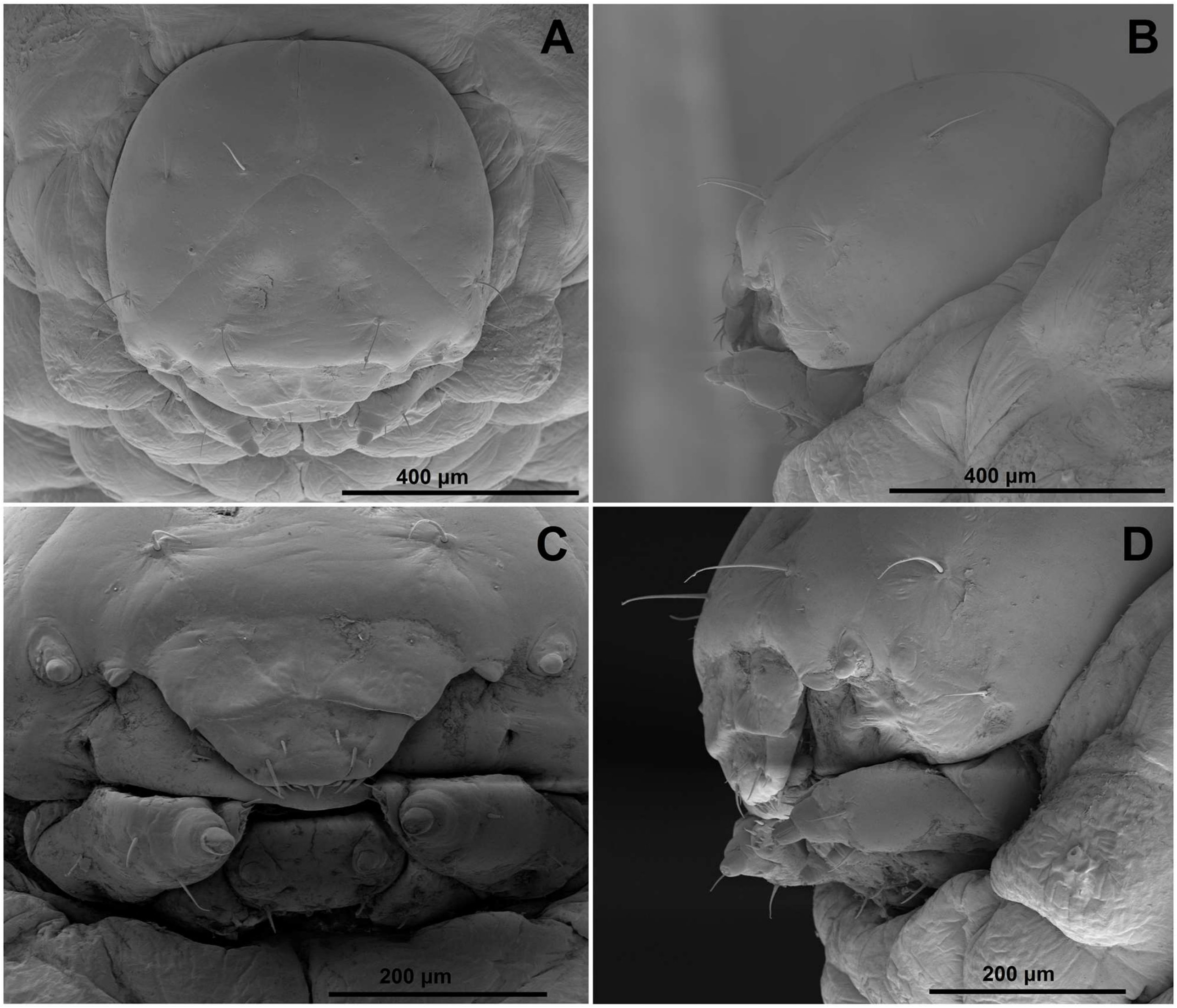

Fig 4. *Eucoeliodes mirabilis*: head and mouthparts of mature larva in SEM image. (A) Dorsal view. (B) Lateral view. (C) Dorsal view of clypeus and labrum. (D) Lateral view of mouthparts.

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almost equal length, next abdominal segments decreasing gradually to the terminal parts of the body. Abdominal segment X reduced to four anal lobes of unequal size; the dorsal lobe distinctly the largest, the lateral pair equal in size, and the ventral lobe very small. Anus located terminally. Spiracles (9 pairs) bicameral, the first placed between the pro- and mesothorax (see Materials and methods), the abdominal spiracles located laterally, close to the anterior margin of abdominal segments I–VIII; all of them have an elongated safety valve protected aperture against possible penetration of faeces inside (see in Chaetotaxy of mature larvae, and Discussion).

Chaetotaxy of mature larva: Chaetotaxy of body very reduced, lower part completely without macrosetae. Setae thin, very short (up to minute) to sometimes short (one relatively long), light yellow or orange. Dorsal part of body up to spiracles covered by microtrichia (Fig 6A–6E, more in Discussion), with an approximate length of 10 µm, but variable. Density of microtrichia high, and space surrounding each was a maximum of ca 10 µm (see Fig 6A–6E).

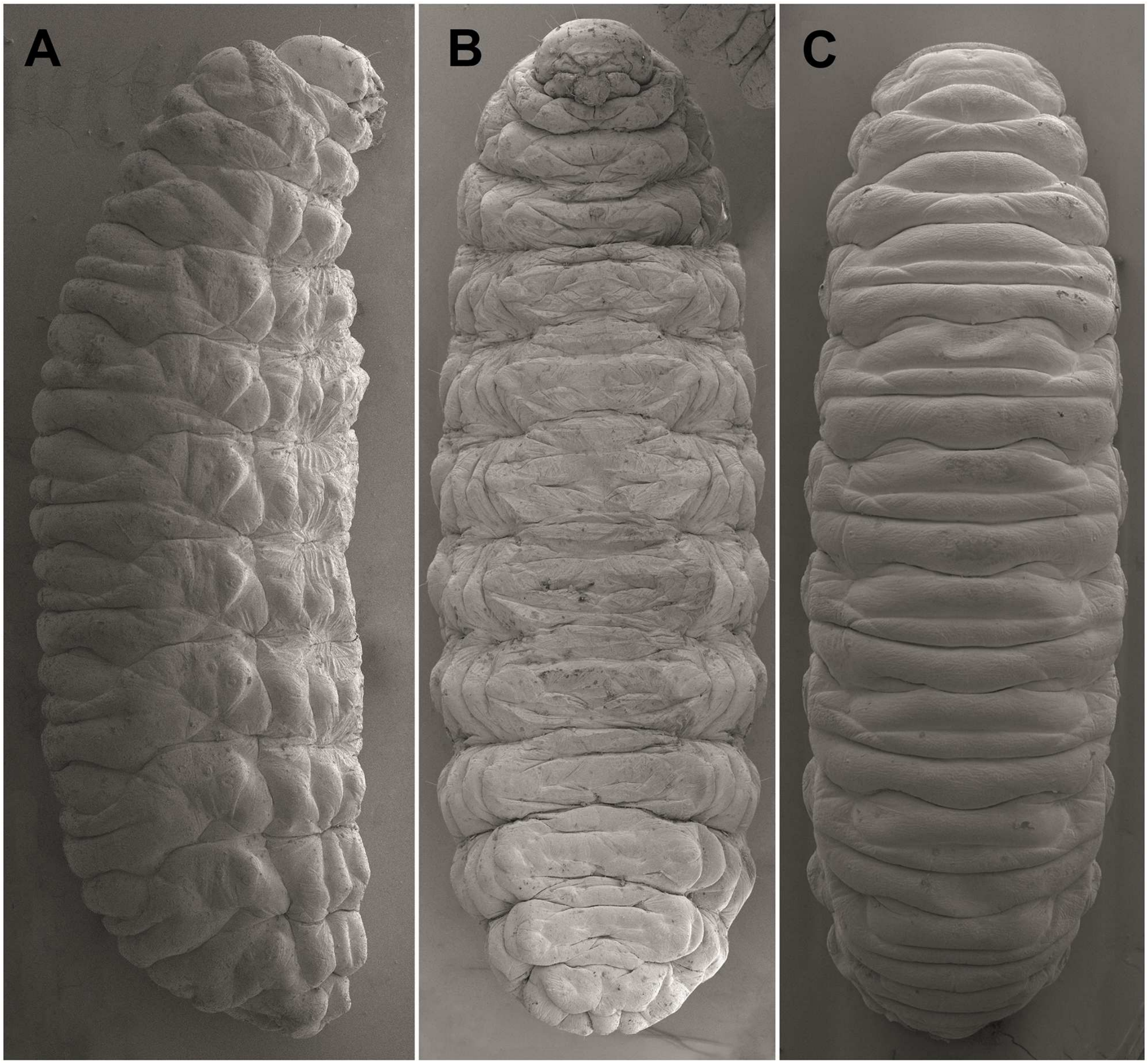

Fig 5. *Eucoeliodes mirabilis*: mature larva in a SEM image. (A) Lateral view. (B) Ventral view. (C) Dorsal view.

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Microtrichia around spiracles distinctly stronger and in higher density, with some clusters (see Fig 7A–7C, more in Discussion).

Thorax: Prothorax (Fig 8A) with 6 *prns* unequal in length (1 relatively long, 1 short, 1 very short and 3 minute), 2 on a weakly pigmented dorsal sclerite subdivided into two triangular plates medially, 4 close to spiracle; and 1 very short *ps*. Mesothorax (Fig 8A) with 2 *pds* unequal in length, *pds1* minute, *pds2* short; and 1 very short *ss*. Chaetotaxy of metathorax (Fig 8A) was identical to that of mesothoracal.

Abdomen: Abdominal segments I–VIII (Fig 8B and 8C) with 5 *pds*, *pds2* very short, remaining 4 setae minute; and 2 *ss* of unequal length, *ss1* minute, *ss2* very short. Abdominal segment IX (Fig 8C) with 2 *ds* (*ds1* minute, *ds2* very short); and 2 *ps* of unequal length, *ps1* very short, *ps2* minute. Abdominal segment X (Fig 8C) without setae.

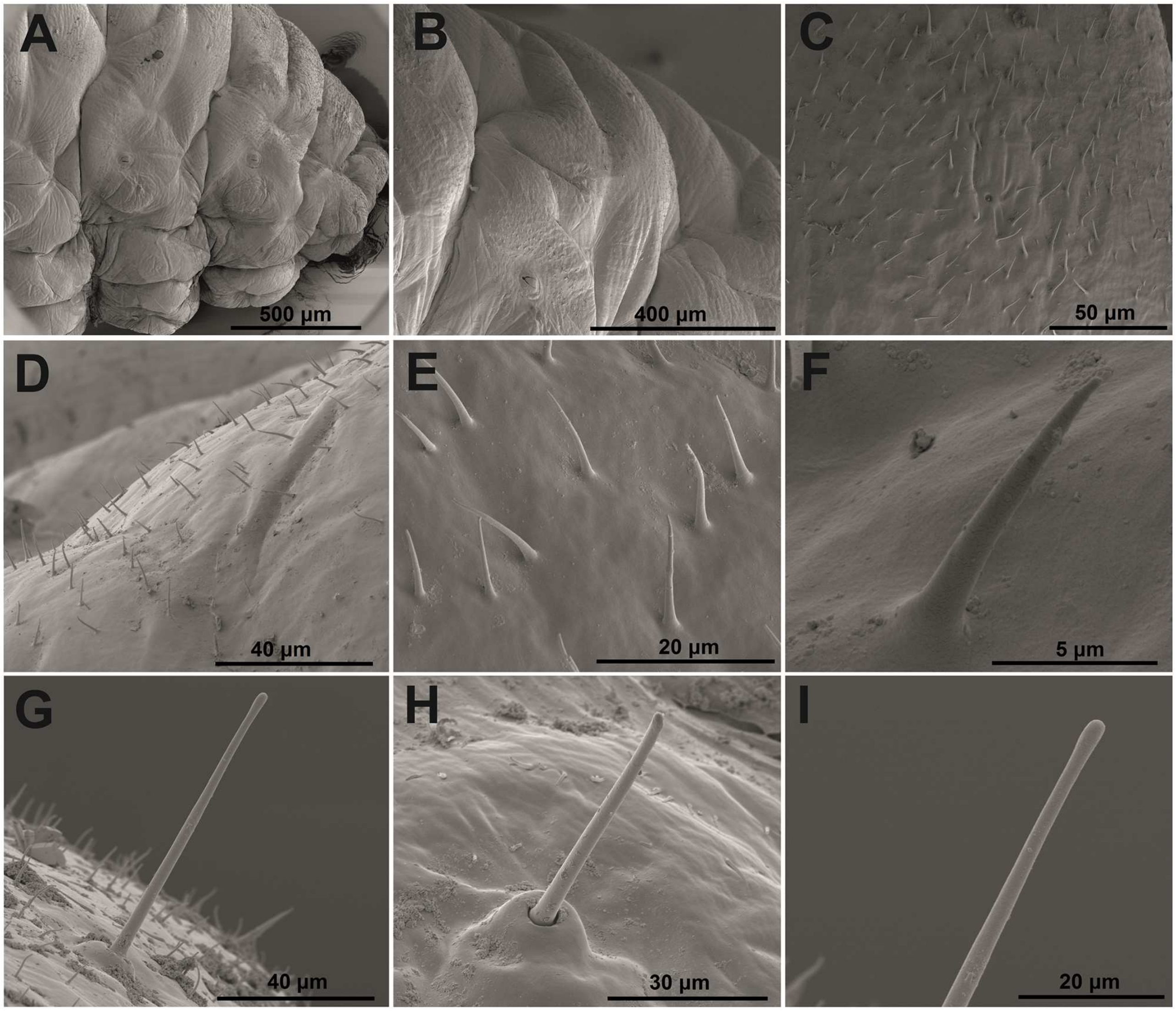

Fig 6. *Eucoeliodes mirabilis*: SEM photos of body with microtrichia and macroseta. (A) Lateral view of thorax at 500 µm. (B) Lateral view of thorax at 400 µm. (C) Microtrichiae at 50 µm. (D) Microtrichiae at 40 µm. (E) Microtrichiae at 20 µm. (F) Microtrichiae at 5 µm. (G) Seta at 40 µm. (H) Seta at 30 µm. (I) Apex of seta at 20 µm.

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Morphology of pupa. Measurements (in mm): Body length: 3.9–4.3 (♂ 3.9–4.2; ♀ 4.0–4.3), at the widest region: 1.8–2.2. The widest place on the body is commonly between the apex of the meso- or metafemora.

Colouration: Body whitish to yellowish.

Morphology (Fig 9A–9C): Body stocky. Cuticle smooth. Rostrum short and wide, approximately 2 times as long as wide, extended to mesocoxae. Antennae relatively long and stout. Pronotum from 1.6 to 1.8 times as wide as long. Mesonotum and metanotum of approximate equal length. Abdominal segments I–IV of almost equal length, abdominal segment V semicircular; next abdominal segments diminish gradually to the end of the body. Abdominal segments VI–IX distinctly smaller than the other abdominal segments. Gonotheca (abdominal segment IX) in females (2 specimens) bilobed. Sexual dimorphism in the weevils is visible

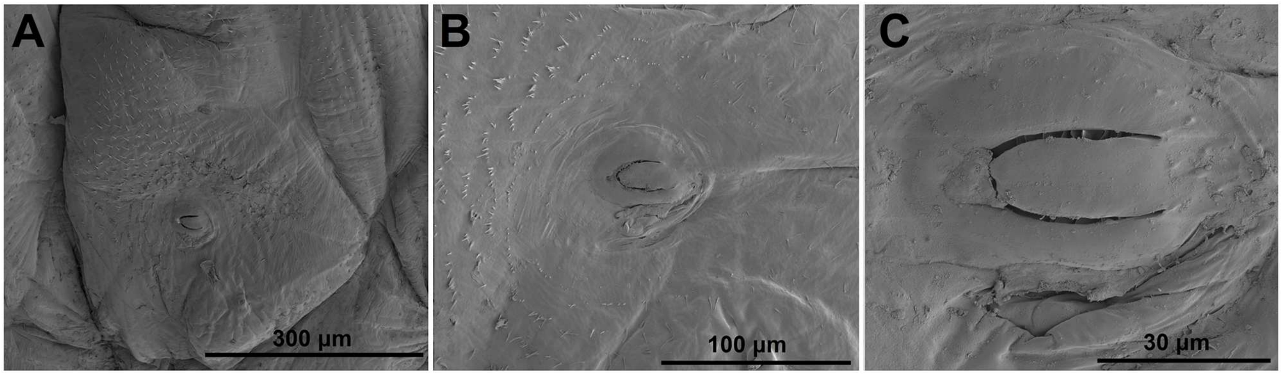

Fig 7. ***Eucoeliodes mirabilis*****: SEM photos of spiracles with microtrichia and valve.** (A) Lateral view of spiracles at 300 μm. (B) Detail at 100 μm. (C) Detail at 30 μm.

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primarily in the length of rostrum and in the structure of abdominal segment IX: gonotheca of ♂ undivided, of ♀ divided.

Chaetotaxy (Fig 9A–9C): Chaetotaxy of body very reduced. Setae relatively long on protuberances, unequal in length, light yellow or orange. Setae well visible. Head capsule included only 1 *vs* and 1 *os*. Rostrum with 1 *rs*. Setae on head capsule straight, as short as the remaining setae on thoracic and abdominal segments. Pronotum with 2 *as*, 1 *ds*, 1 *ls* and 3 *pls*, *as2* distinctly shorter than the remaining setae. Dorsal parts of meso- and metathorax with 1 seta

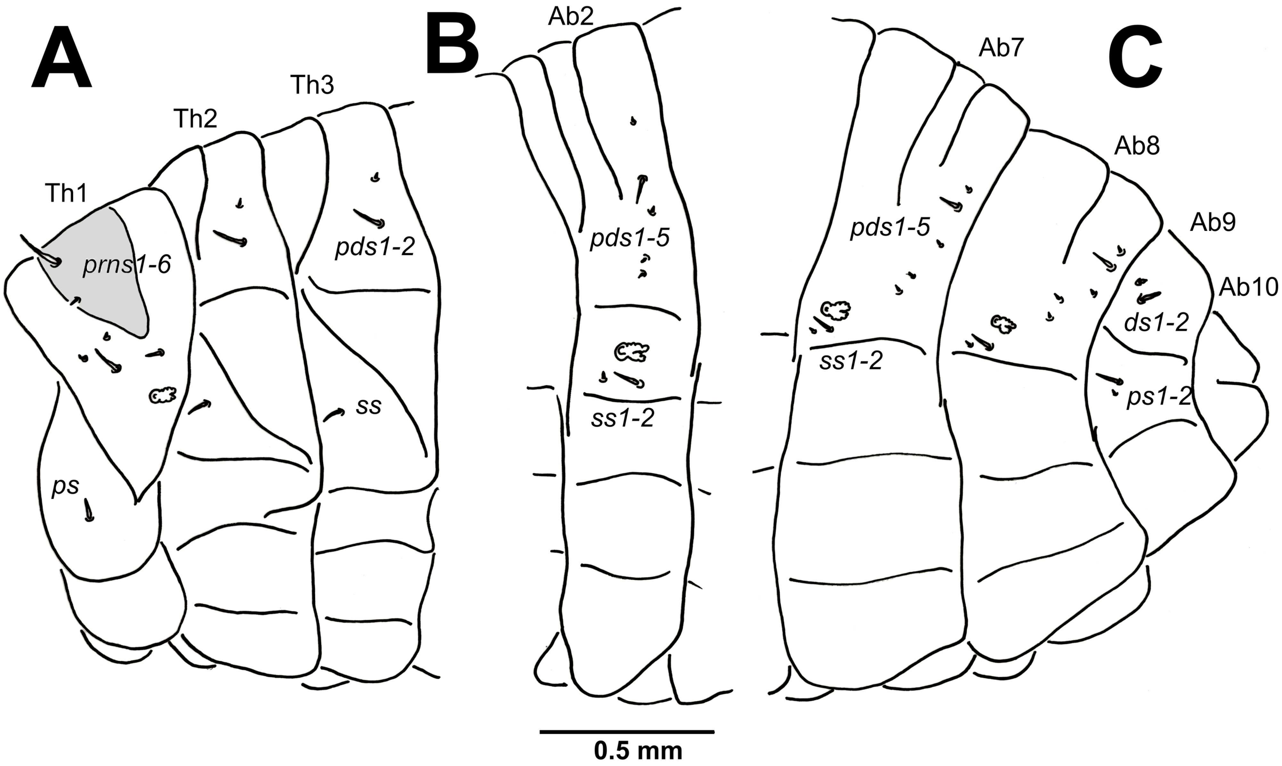

Fig 8. ***Eucoeliodes mirabilis*****: mature larva.** (A) Lateral view of thoracic segments. (B) Lateral view of abdominal segment II. (C) Lateral view of abdominal segments VII–X. Abbreviations: Ab.–abdominal segment, Th.–thoracic s., I–X—number of segments, *prns*–pronotal seta (e), *pds*–postdorsal s., *ps*–pleural s., *ss*–spiracular s., and *ds*–dorsal s.

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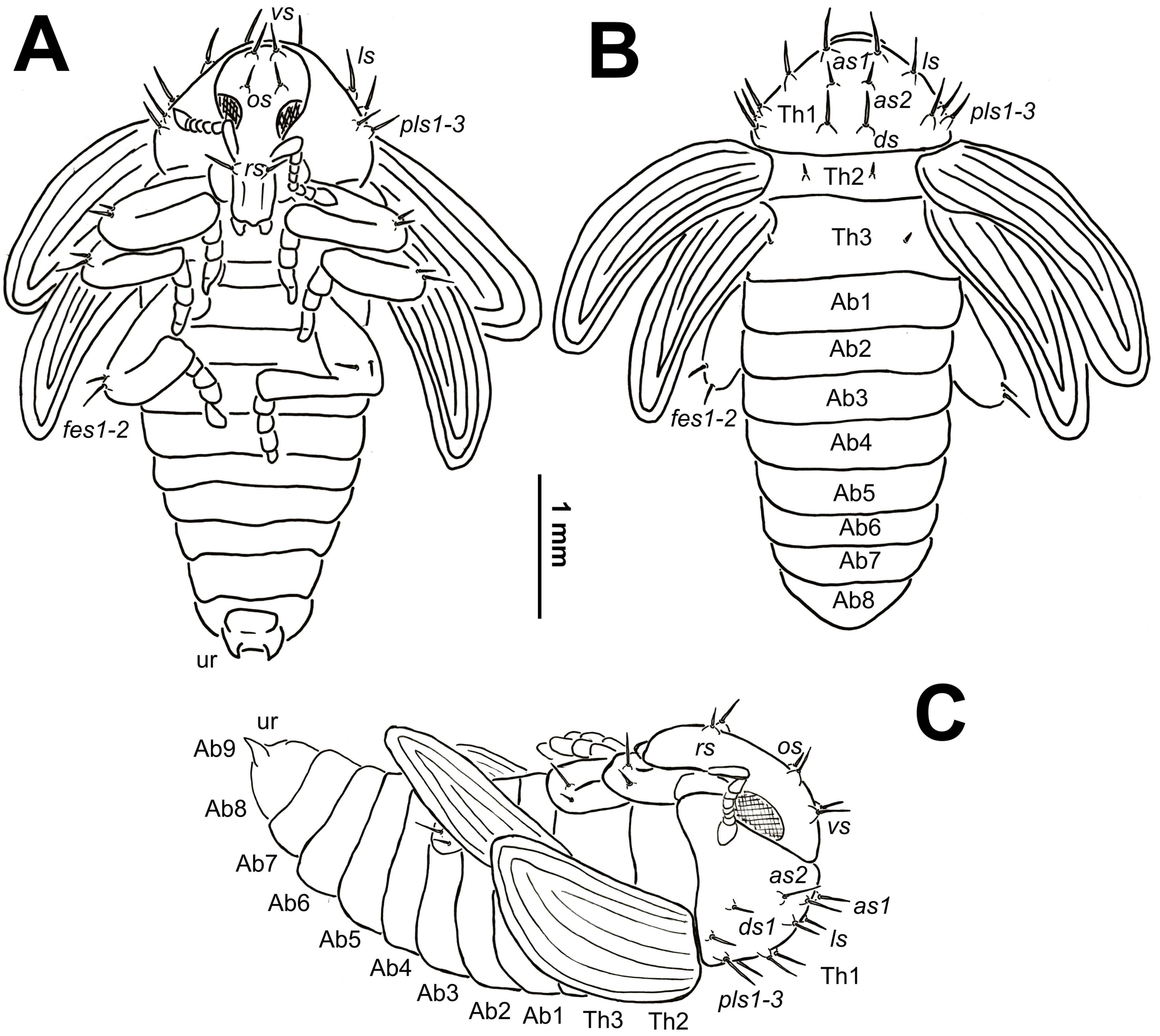

Fig 9. *Eucoeliodes mirabilis*: pupa habitus. (A) Ventral view. (B) Dorsal view. (C) Lateral view. Abbreviations: *vs*–vertical seta(e), *rs*–rostral s., *as*–apical s., *ls*–lateral s., *ds*–discal s., *pls*–posterolateral s., and *fes*–femoral s.; Th1-3 and Ab1-9–number of thoracic and abdominal segments, respectively, and ur—urogomphi.

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located posterolaterally. Each apex of femora with groups of 2 *fes*. All abdominal segments I–VIII without setae. Urogomphi short, strong, triangular.

Discussion

Defensive strategies in ectophagous weevil larvae

Weevil larvae (Coleoptera: Curculionoidea) typically lead an endophytic life; however, some weevil groups live ectophytically [32]. Apart from the speciose subfamily Entiminae (more than 12 000 species in 1 370 genera) [40] with ectophytic but endophagous larvae (living in soil among roots), only a few species have both ectophytic and ectophagous larvae. Endophytic/endophagous larvae, either living in soil or inside plant tissues, are legless and largely sedentary, whereas ectophytic and ectophagous larvae live exposed on aerial parts of plants and have adaptations for moving on plant surfaces, including ambulatory ampullae, pedal lobes and creeping soles [32]. The ectophagous and ectophytic larvae are found in only a few groups, primarily most members of the subfamily Cyclominae [32, 41–43] and the unclassified tribes Bagoini, Gonipterini and Hyperini [32, 41, 43, 44]. These insects have all evolved strategies of physical or chemical defence against predators and parasitoids [45–48]. In Central Europe, in which this new defensive strategy in weevils was observed, ectophagous larvae, feeding on leaves or flowers, are currently found only in the following tribes: Bagoini, Cionini, Hyperini, Phytobiini and rarely Ceutorhynchini [32, 36, 49].

These known ectophagous larvae in Central Europe have three primary defensive strategies against predators and parasitoids. (1) The Hyperini are a well-known group of weevils with ectophagous larvae, which rely on cryptic coloration [45]. Members of the genus *Phelypera* from this tribe also benefit from the defensive strategy known as cycloalexy (a form of gregariousness that involves group reactions, see [46]). (2) Living in relatively unusual habitats is also a strategy to avoid predators and parasitoids because the pressure of parasitoids is partially decreased (e.g., Bagoini and Phytobiini live in aquatic habitats, and some Entiminae, e.g., *Otiorhynchus*, live in soil) [47]. The weevil tribe Cionini uses a different strategy (3) in which viscid mucus covers and protects larval bodies [48], but this mucus is primarily protection from desiccation [50].

Larvae of the subfamily Ceutorhynchinae live primarily endophytically [51], and the only known exception is *Ranunculiphilus faeculentus* (Gyllenhal, 1837), with ectophytic larvae that feed on terminal buds of *Consolida regalis* Grey (Ranunculaceae) [52]. Our biological investigations showed that all larval stages of *E. mirabilis*, which is also in the tribe Ceutorhynchini, feeds ectophytically on leaves and used a faecal shield for covering the entire dorsum. The shield is obviously green when squeezed and is most likely of vegetal origin. We hypothesized that the shield played a defensive role but also prevented desiccation.

To date, very little is known about the biology of the weevil *E. mirabilis*, which is also the case for species of the closely related genus *Coeliodes* Schoenherr, 1837. The life history is well documented only for another close relative, *Pseudocoeliodes rubricus* (Gyllenhall, 1837), in which the larvae develops in male flowers of *Pistacia* [53]. This species is univoltine and overwinters as an adult. In spring, adults feed on host plants. Larvae develop in male flowers and floral axes and pupate in soil at the end of April/beginning of May. Adults of the new generation aestivate after a short feeding [53]. Based on this information, many previous authors reached the obvious conclusion that larval development in flowers or flower buds is likely for the genera *Coeliodes*, *Coeliodinus* Dieckmann, 1972 and *Eucoeliodes* (all Coleoptera: Curculionidae: Ceutorhynchini) [27, 30, 49], although this type of development has never been supported by direct observation, including in our field study. Larval feeding marks of *E. mirabilis* closely resembled those of *Steronychus fraxini* (DeGeer, 1775) (Coleoptera: Curculioninae: Cionini), which feeds on the leaves of *Fraxinus* (R. Stejskal, pers. observ.).

Morphological adaptations to fix the faecal shield in beetles

Currently, faecal ecology in beetles is known only for the immature stages of leaf beetles. Within Chrysomelidae, three primary defensive strategies use faeces with morphological adaptations: 1, super-anal processes to carry defensive shields composed of shed larval skins (exuviae) and faeces that are often retained after pupation (Cassidinae); 2, portable enclosures of bell-shaped cases of faeces and also plant material (Lamprosomatinae and Cryptocephalinae); and 3, accumulation of faeces on the dorsum to partially or entirely cover the larvae (Criocerinae and Blepharida-group Galerucinae).

Using faeces as a defensive shield in *E. mirabilis* is a strategy similar to that in the Criocerinae. The faecal shield in this group is a physical and also probable chemical barrier against predators and parasitoids, but compared with the Criocerinae, different morphological adaptations have developed in *E. mirabilis*. Only a few Criocerinae larvae [18, 54] are well described, and the knowledge about the variability of their morphological characters is very poor. Anyway, it is known that the dorsal vestiture of a few known Criocerinae larvae is covered by short, relatively sparse setae [18, 54] unlike that of *E. mirabilis*, whose macrosetae are completely reduced and the dorsal vestiture is covered with a high density of microtrichia (Fig 6A–6E). Based on the character and arrangement, these microtrichia likely act effectively as a velcro. Because of the high density of microtrichia on the dorsal vesture, the faecal shield is most likely removed only during moulting, in contrast to the Criocerinae, which have short and sparse setae on the dorsum [18] and the faecal shield is easily removed by unfavourable conditions.

Moreover, distinct morphological adaptations for protecting spiracles against faeces have also been found on *E. mirabilis* larvae. The density of microtrichia around all spiracles was high, and the microtrichia are in clusters and are slightly shorter and distinctly stronger. These morphological differences all contributed to the protection from and the prevention of faecal incursion into the tracheal system. However, the microtrichia is not the only morphological adaptation, and all spiracles are also protected by a safety valve, which reduces the space in which faeces might penetrate. Spiracles of Criocerinae larvae are minute but distinct [54], unlike those of *E. mirabilis*, which are protected by the altered microtrichia and safety valve (Fig 6A–6E). These morphological adaptations, including velcro on the dorsal vesture, the system of protection around spiracles and the safety valve in spiracles, are all unique, and these adaptations were used in combination to use faeces as a defensive strategy in *E. mirabilis*.

Comparison with immature stages of other Ceutorhynchinae species

The larvae of 58 Ceutorhynchinae taxa in 22 genera have been described previously [36, 51, 55–65]. A detailed description of the pupa is known for only six Ceutorhynchinae taxa [36, 56, 62, 63]. The immature stages were compared with most of the species described or drawn by Anderson [56, 57], Scherf [36], May [59], Lee and Morimoto [60], Orlova-Bienkowskaya [61], Gosik [62–64] and Nikulina [65]; these illustrations are all of high or sufficient quality and therefore were very useful; however, the described characteristics are useful only for differential diagnosis. The comparison of the larvae and pupae with some species described by Scherf [36] was somewhat difficult due to the use of different terminology for morphology and chaetotaxy and/or the absence of good-quality drawings and data about some important morphological features. In that case, the comparison with such taxa has not been possible, and they cannot be included in the *Key to the immatures of the subfamily Ceutorhynchinae*.

The most precise general description of larvae of the subfamily Ceutorhynchinae, which is summarized by 9 character sets, is presented by May [59]: (1) head with *des3* on epicranial half (Fig 3A); (2) antennae hemispherical, fully exposed (Fig 3A and 3D); (3) endocarinal line absent (Fig 3A); (4) frons only with *fs4* developed (Fig 3A); (5) tormae separate, subparallel (Fig 3F); (6) postlabium with proximal pairs of *plbs* as far apart as the median pair (Fig 3B); (7) abdominal spiracles with air-tubes caudad (Fig 8B and 8C); (8) abdominal VIII spiracle lateral (Fig 8C); and finally (9) abdominal segments with ventral lobes developed as ambulatory ampullae (Fig 5A–5C). Almost all these characters fit with known descriptions [36, 51, 55–65]. There is observed only one exception, the statement number 4 –frons only with *fs4* developed. The larvae of *E. mirabilis* has frons with long *fs4*, but is has also three other short to minute setae (*fs1*, *fs3* and *fs5*) (see Fig 3A). This feature is not consistent with other descriptions, e.g., by Anderson [57] (*Ceutorhynchus rapae* Gyllenhal, 1837; larvae have short, but distinct *fs5*), Orlova-Bienkowskaya [61] (*Phytobius leucogaster* (Marsham, 1802) (published as *Litodactylus lecuogaster*) with 6 *fs*), Gosik [63] (*Tapeinotus sellatus* (Fabricius, 1794) with 3 *fs*), Gosik [64] (*Mogulones austriacus* (Ch. Brisout de Barneville, 1869) and *M. dimidiatus* (Frivaldszky, 1865) has 4 *fs*), and Nikulina [65] (*C. subtilirostris* Schultze, 1902 and *C. viator* Faust, 1885; larvae have short, but distinct *fs1*).

Macrosetae on larval and also pupal bodies of *E. mirabilis* are completely reduced (Figs 8A–8C and 9A–9C). All described morphological adaptations in *E. mirabilis*, including velcro on the dorsal vesture, the system of protection around spiracles and the safety valve in spiracles (more in previous chapter), are absolutely unique not only in the subfamily Ceutorhynchinae, but also completely in all weevils and probable also in all beetles. Larvae of *E. mirabilis* have also some other unique characters in the subfamily Ceutorhynchinae as follows; (1) trifid mandible (Fig 3C), and (2) only two *ams* and also two *mes* on epipharynx (Fig 3F). All known larvae of Ceutrohynchinae have only bifid mandibles, and three *ams* and two *mes* on epipharynx. The position of absent *ams* seta is often problematic. According to Marvaldi [38, 39], the standard status of the epipharynx in weevils is 2 *ams* and 3 *mes*, but when the position of the distal *mes* is very close to the anterior margin, they appear as *ams*. The decision was finally made to add this problematic seta to the latter group (*ams*), and the position of this seta is similar to that in other genera, e.g., in *Coniocleonus* Motschulsky, 1860 or *Tychius* Germar, 1817. We did not follow Stejskal et al. [66] and Skuhrovec et al. [67], who accepted the standard status in weevils and counted the seta as *mes*, but we followed Trnka et al. [68] and Skuhrovec et al. [69], e.g., in *Adosomus* Faust, 1904 or *Sibinia* Germar, 1817. Count of setae on ventral side of maxilla (*vms*) is also different from other species, but this is not a unique feature, and in this case, there are probably many mistakes in descriptions as for example descriptions of two *Poophagus* species [62], where probably 2 *vms* are counted as *dms* in *Poophagus hopffgarteni* Tournier, 1873.

Key to the immatures of the subfamily Ceutorhynchinae

Larvae. The following key is based on a recent description of immature stages in the genus *Eucoeliodes* and descriptions of immature stages published before: *Ceutorhynchus* (8 species) [36, 55, 57, 60, 65]; *Eubrychius* (1 species) [61]; *Glocianus* (1 species) [36]; *Homorsoma* (1 species) [60]; *Hypurus* (1 species) [60]; *Mecysmoderes* (1 species) [60]; *Mogulones* (5 species) [58, 64]; *Phrydiuchus* (2 species) [56]; *Phytobius* (1 species) [61]; *Poophagus* (2 species) [62]; *Rhinoncus* (2 species) [59, 60]; *Tapeinotus* (1 species) [63]; and *Trichosirocales* (1 species) [59]. Unfortunately, some basal data about chaetotaxy on body is missing, and then it is not possible to distinguish all genera (see accumulation of genera in point 6). Further comments are reported in the previous chapter in Discussion.

1. Head broadly and deeply emarginate at posterior margin. .... ***Hypurus***

-. Head normally rounded posteriorly. **2**

2. Eighth abdominal spiracles on papillae. Air-tubes of spiracles different in length, dorsal air-tube about half as long as ventral air-tube. .... ***Mecysmoderes***

-. Eighth abdominal spiracles not on papillae, but near dorsal bases of papillae or in normal position. **3**

3. Dorsal part of body covered by vestitutre with many microtrichia. .... ***Euceoliodes***

-. Dorsal part of body covered by vestitutre without any microtrichia. **4**

4. Frons with 5 or more *fs*. .... ***Phytobius***

-. Frons with 4 or less *fs*. **5**

5. Dorsal epicranium with 5 *des*. .... ***Tapeinotus***

-. Dorsal epicranium with less than 5 *des*. **6**

6. Dorsal epicranium with 3 *des*. **7**

-. Dorsal epicranium with 4 *des*. ....
..................***Ceutorhynchus*, *Homorsoma*, *Mogulones*, *Phrydiuchus*, *Poophagus*, *Trichosirocales***

7. Abdominal segments I–VII with 4 *pds*. .... ***Rhinoncus***

-. Abdominal segments I–VII with less than 4 *pds*. **8**

8. Abdominal segments I–VII with 3 *pds*. .... ***Eubrychius***

-. Abdominal segments I–VII with 1 *pds*. .... ***Glocianus***

Pupae. The following key is based on recent description of pupa in the genus *Eucoeliodes* and descriptions of pupae published before: *Glocianus* (1 species) [36]; *Phrydiuchus* (2 species) [56]; *Poophagus* (2 species) [62]; and *Tapeinotus* (1 species) [63].

1. Rostrum elongated, at least 5 times as long as wide. **2**

-. Rostrum short and wide, about 2 times as long as wide. .... ***Eucoeliodes***

2. *Pas* present. **3**

-. *Pas* absent. **4**

3. Meso- and metathorax and also abdominal segments without seta. Femora with 1 seta. ...
.... ***Tapeinotus***

-. Meso- and metathorax with 1 seta, and abdominal segments with more than 3 setae on dorsal part. Femora with 2 setae. .... ***Phrydiuchus***

4. Rostrum very slender, 8 times as long as wide, *sos* absent. .... ***Poophagus***

-. Rostrum elongated, 5 times as long as wide, *sos* present. .... ***Glocianus***

Conclusions

Our observations confirm that the larvae of *E. mirabilis* feed exclusively ectophytically on leaves. The appearance of *E. mirabilis* larvae on leaves is remarkable, with the protective faecal shield covering the entire body. Although most similar to the strategy adapted by the Criocerinae, the defensive shield as a strategy is unique in *E. mirabilis*, and the larvae have clearly different morphological adaptations. Macrosetae on larval and also pupal bodies of *E. mirabilis* are completely reduced, and the vesture is covered with a high density of microtrichia, which serve as velcro. Because of the high density of microtrichia on the dorsal vesture, the faecal shield is most likely removed only during moulting. As a system to protect spiracles against incursion of faeces into the tracheal system, the density of microtrichia around all spiracles is high, and the microtrichia are slightly shorter, distinctly stronger, and arranged in clusters. All spiracles are also protected by a safety valve, which decreases the space in which faeces could enter. All these mentioned morphological adaptations are unique and used in combination to use faeces as a defensive strategy in *E. mirabilis*. Finally, the generic keys for identification of larvae and pupae based on useful preliminary descriptions is also presented. All these new data about biology and also morphology of *E. mirabilis* are very useful in next studies of immature stages in Polyphaga beetles, but there are also still some missing gaps (e.g. chemical composition of host plant tissues, and feces; description of shield construction; morphology of younger instars, etc.).

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Author Contributions

Conceptualization: JS RS FT.

Investigation: RS FT.

Methodology: JS AG.

Project administration: JS.

Resources: JS RS FT AG.

Supervision: JS RS FT AG.

Visualization: JS RS FT AG.

Writing – original draft: JS RS.

Writing – review & editing: JS RS FT AG.

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PŘÍLOHA VI

Prohlášení spoluautorů o autorském podílu uchazeče na publikacích.

Autorský podíl Filipa Trnky na publikaci:

Trnka F., Stejskal R., Šipoš J., Kuras T. (2019): Weevils: suitable candidates for prediction of herbivorous insect extinction risk? Submitted to *Biological Conservation*. (IF = 4.660)

Filip Trnka se podílel na designu studie, tvorbě datasetu, interpretaci výsledků, psaní manuskriptu, revizích a korekturách textu.

Jeho procentuální podíl tedy činí přibližně 40 %.

Spoluautoři svým podpisem stvrzují pravdivost uvedeného podílu Filipa Trnky na publikaci:

Stejskal Robert

Šipoš Jan

Kuras Tomáš

Autorský podíl Filipa Trnky na publikaci:

Stejskal R., Trnka F. & Skuhrovec J. (2014): Biology and morphology of immature stages of Coniocleonus nigrosuturatus (Coleoptera: Curculionidae: Lixinae). *Acta Entomologica Musei Nationalis Pragae* 54: 337–354. (IF = 0.659)

Filip Trnka se podílel na designu studie, na sběru dat, interpretaci výsledků, psaní manuskriptu, revizích a korekturách textu a zapracovával připomínky recenzentů.

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Stejskal Robert

Skuhrovec Jiří

Autorský podíl Filipa Trnky na publikaci:

Trnka F., Stejskal R., Skuhrovec J. (2015): Biology and morphology of immature stages of Adosomus roridus (Coleoptera: Curculionidae: Lixinae). *Zootaxa* 4021: 433–446. (IF = 0.994)

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Autorský podíl Filipa Trnky na publikaci:

Trnka F., Stejkal R., Skuhrovec J. (2016): The morphology of the immature stages of two rare Lixus species (Coleoptera, Curculionidae, Lixinae) and notes on their biology. *ZooKeys* 604: 87–116. (IF = 1.031)

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Skuhrovec Jiří

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Ekologie herbivorních a saproxylických brouků (Coleoptera)

Filip Trnka

Autoreferát dizertační práce

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Uchazeč:	**Mgr. Filip Trnka** Přírodovědecká fakulta Univerzity Palackého v Olomouci Katedra ekologie a životního prostředí
Školitel:	**prof. MVDr. Emil Tkadlec, CSc.** Přírodovědecká fakulta Univerzity Palackého v Olomouci Katedra ekologie a životního prostředí
Konzultant:	**RNDr. Tomáš Kuras, Ph.D.** Přírodovědecká fakulta Univerzity Palackého v Olomouci Katedra ekologie a životního prostředí
Oponenti:	**doc. Ing. Jan Bezděk, Ph.D.** Agronomická fakulta Mendlovy zemědělské a lesnické univerzity Ústav zoologie, rybářství, hydrobiologie a včelařství
	doc. RNDr. Pavel Saska, Ph.D. Výzkumný ústav rostlinné výroby, v.v.i. Funkce biodiverzity bezobratlých a rostlin

Obhajoba dizertace se koná dne .... v .... hodin v učebně .... na Katedře ekologie a životního prostředí PřF Univerzity Palackého v Olomouci, Šlechtitelů 27, Olomouc.

S dizertační prací je možné se seznámit na Studijním oddělení Děkanátu PřF Univerzity Palackého v Olomouci, tř. 17. listopadu 12, Olomouc.

ABSTRAKT

Trend poklesu biodiverzity je v současné době nezvratně dokázaným faktem. Abychom byli schopní tento pokles zastavit nebo alespoň zpomalit, je nezbytně nutné znát podrobně jak biologii a ekologii druhů, tak na základě relevantních informací potom umět tvořit modely, které nám mohou pomoci předvídat, které druhy mohou být v budoucnu náchylnější k vymření.

První část této práce si klade za cíl predikovat ohrožení herbivorních brouků na skupině nosatcovitých (Curculionoidea) na základě jejich life history a také ekologických vlastností jejich živných rostlin. Analýza odhalila, že mezi nejvíce ohrožené druhy patří monofágní, nelétavé, teplomilné, světlomilné, suchomilné a hygrofilní druhy s vazbou na otevřené biotopy. Obdobně je to i s vlastnostmi živných rostlin, které vysvětlují ohrožení nosatcovitých. Tyto rostliny lze charakterizovat jako xerotermofilní a hygrofilní vyskytující se na biotopech s minimem dusíku. Z analýzy dále vyplývá, že nosatci, jejichž živné rostliny jsou obecně na ústupu a mají nízkou abundanci na stanovišti, jsou více náchylní k vyhynutí. A v neposlední řadě jsou ohroženější nosatci s vazbou na vodní rostliny a keříčkovitou vegetaci. Life history charakteristiky nosatců se ze všech studovaných vlastností ukázaly pro predikci ohrožení důležitější než ekologické vlastnosti rostlin. Nicméně vlastnosti živných rostlin se zdají být důležité a měly by být použity pro predikční modely herbivorních skupin živočichů (Příloha I).

Druhá část práce se zaměřila na základní výzkum a odhaluje bionomii několika druhů nosatců, jejich živné rostliny, larvální stádia, stanovištní preference, ekologické a morfologické adaptace. Tyto výsledky významně přispívají k základnímu výzkumu, ale budou mít i praktické důsledky pro ochranářskou biologii a mohou být využity k biologické kontrole invazních druhů rostlin (Příloha II–V).

Klíčová slova: Coleoptera, Curculionoidea, larvální stádia, life history, morfologie, nosatci, ochrana přírody, riziko vyhynutí, živné rostliny

ABSTRACT

Nowadays biodiversity decline is the indisputable fact. To stop or at least slow down this trend a detailed knowledge of species biology and ecology is essential. Consequently, the relevant biological information may help us create models for prediction of species extinction risk in the future.

First part of this doctoral thesis is focused on the prediction of the extinction risk of the herbivorous beetles from the weevil group (Curculionoidea) based on their life history traits and the ecological traits of their host plants. Our analysis revealed the most endangered species are the monophagous and flightless ones that belong to the thermophilic, heliophile, xerophilous or hygrophilous species of open habitats. For the ecological host plant traits explaining the extinction risk of the weevils the situation is almost the same. Those plants could be ranked among xerothermophilous and hygrophilous species occuring on the low nitrogen biotopes. According to our analysis weevils whose host plants are declining and have low abundances at their biotopes are more vulnerable to the extinction risk. Also the weevils connected to aquatic plants and shrubby vegetation are more vulnerable. Weevil life history traits were proved to be more imporant factor for the prediction of the extinction risk than the ecological host plant traits. Nevertheless, the ecological host plant traits are also important and should be used for the prediction models od the herbivore species groups (Appendix I).

Second part of this doctoral thesis is focused on the basic research of the bionomy of a few weevil species, their host plants, larval stages, habitat preferences and morphological and ecological adaptations. The results significantly contribute to the basic knowledge about these species but they may also have a practical use in conservation biology and biological control of invasive plants (Appendices II-V).

Key words: Coleoptera, Curculionoidea, extinction risk, host plants, immature stages, life history, morphology, nature conservation, weevils

OBSAH

ÚVOD

Celosvětový pokles biodiverzity je už po desítky let aktuálním tématem (Thomas & Abery 1995, Pimm & Raven 2000). Teprve v posledních letech je ale diskutován i významný pokles biodiverzity bezobratlých a obzvláště hmyzu (Sánchez-Bayo & Wyckhuys 2019). Ochrana biologické rozmanitosti na Zemi je mezinárodním zájmem (*Convention on Biological Diversity*; CBD 2014). Tuto úmluvu podepsali zástupci všech států světa, pro které je následně ochrana všech živých organismů a jejich prostředí zavazující. Masivní úbytek druhové diverzity, který ve 21. století zažíváme (Butchart et al. 2010, Sánchez-Bayo & Wyckhuys 2019), se neprojevuje pouze poklesem či ztrátou populací vzácných a ohrožených druhů, ale také rychlým ochuzováním diverzity regionální a lokální (Sax & Gaines 2003, Edgar & Samson 2004) a snižováním abundancí běžných druhů (Hallmann et al. 2017).

OHROŽENÍ A OCHRANA HMYZU

Hlavními příčinami ohrožení hmyzu je izolace, fragmentace a destrukce biotopů (Fischer & Lindenmayer 2007), také jejich znečištění (Dudley & Alexander 2017) a v menší míře také globální změna klimatu (Thomas et al. 2004, Urban 2015). Tyto příčiny jsou bezprostředně způsobovány růstem lidské populace a její činností, především intenzivním hospodařením, které se v posledních desetiletích násobně stupňuje (Krebs et al. 1999). Vyjma samotné intenzifikace zemědělství a lesnictví je velkým problémem upouštění od tradičních forem hospodaření, a tím pádem ztráta heterogenní krajiny (Benton et al. 2003). Zejména v případě nelétavých druhů je homogenní krajina s izolovanými a fragmentovanými biotopy neprostupná a snižuje se šance na rekolonizování vhodných biotopů (Thomas et al. 2001). Upouštěním od extenzivní seče a pastevectví se stávají velmi ohrožené různé typy přirozených a polopřirozených otevřených biotopů (Fischer et al. 1996, Dover et al. 2003). Také pastva v lesích nebo výmladkové hospodaření vytváří vhodné podmínky pro dnes ohrožené druhy (Saarinen et al. 2005, Vodka et al. 2009, Horák et al. 2012). Fakticky tedy často vymírají i specializované druhy, které jsou naopak závislé na extenzivní nebo specifické hospodářské činnosti člověka, kterou vznikají typické mikro a makrohabitaty (Roslin et al. 2009, Thomas et al. 2009).

PREDIKCE OHROŽENÍ

Pochopením hlavních příčin úbytku biodiverzity je nezbytné předem predikovat, jaké druhy budou v budoucnu ohroženy. Na pochopení těchto mechanizmů stojícími za snižováním diverzity je zapojeno mnoho faktorů (Fischer et al. 2003), jak vnějších (např. ztráta biotopů, změna klimatu), tak vnitřních (life history). Druhy, které mají podobné life history, mají také pravděpodobně podobnou citlivost vůči změnám v prostředí a také mají podobnou šanci na riziko vymření (Moretti et al. 2017).

Většina studií se soustředí na predikci ohrožení analýzami červených seznamů a vlastností druhů a jsou zaměřeny především na obratlovce (cf. Purvis et al. 2000, Krüger & Radford 2008) nebo poměrně dobře prostudované či charismatické skupiny hmyzu jako jsou denní motýli, vážky, motýlice, saproxyličtí brouci nebo střevlíci (Kotiaho et al. 2005, Clausnitzer et al. 2009, Seibold et al. 2015, Nolte et al. 2019). Tyto výsledky jsou proto jen částečně přenosné na další skupiny hmyzu a mohou být zkreslené, proto je tedy nutné studovat i další skupiny bezobratlých.

V rámci naší studie k predikci ohrožení herbivorních skupin hmyzu (Příloha I.) jsme jako vhodnou skupinu vybrali nosatcovité brouky (Curculionoidea), kteří jsou považovaní za indikátory zachovalosti prostředí (Benedikt et al. 2010) a jedná se zdaleka o největší skupinu herbivorů (Oberprieler et al. 2014). Ve studii jsme testovali nejen life history téměř 1 000 druhů nosatcovitých ale také ekologické vlastnosti jejich živných rostlin (Ellenberg et al. 1992) společně s analýzou červeného seznamu nosatců (Benedikt & Strejček 2005). Fylogeneticky upravená analýza (Felsenstein 1985, Harvey & Pagel 1991) nám umožnila identifikovat life history charakteristiky, které vedou k poklesu populací a predikují, které druhy mohou být potenciálně ohroženy.

Naše analýza odhalila, že mezi herbivorními druhy nosatců patří k nejvíce ohroženým skupinám ty druhy, které jsou monofágní, nelétavé, teplomilné, světlomilné, suchomilné a vlhkomilné a žijící na otevřených stanovištích. Z živných rostlin nejlépe vystihují ohroženost druhů nosatců ty, které jsou suchomilné a teplomilné nebo mokřadní s vazbou na otevřené biotopy. Z analýzy dále vyplývá, že nosatci, jejichž živné rostliny jsou obecně na ústupu, jsou vzácné a mají nízkou abundanci na stanovišti, jsou více náchylní k vyhynutí. A nakonec hydrofyty (vodní rostliny) a chamaefyty (polokeře) předurčují více ohrožené nosatce. V celkové analýze všech používaných charakteristik se ukázalo, že life history charakteristiky druhů nosatců jsou pro predikci ohrožení více důležité než ekologické vlastnosti jejich živných rostlin. Zároveň jsou ale ekologické charakteristiky živných rostlin důležité a měly by v modelech predikce ohrožení herbivorních druhů být jednoznačně používány dohromady s charakteristikami herbivorů.

NOSATCOVITÍ BROUCI

Nadčeleď nosatcovitých brouků (Curculionoidea) je celosvětově nejpočetnější a nejdiverzifikovanější skupinou brouků, pravděpodobně i veškerého hmyzu (Oberprieler et al. 2014). Počet popsaných taxonů je odhadován na 100 000 (Astrin et al. 2012), celkové odhady jsou okolo 220 000 druhů (Oberprieler et al. 2007). Diverzita nadčeledi bude pravděpodobně i násobně větší, jelikož v poslední době bylo popsáno několik velmi diverzifikovaných kryptických rodů s desítkami druhů (cf. Anderson 2010, Riedel 2010). V moderních fylogenetických klasifikacích se nadčeleď skládá ze sedmi čeledí – Nemonychidae, Anthribidae, Belidae, Attelabidae, Caridae, Brentidae a největší čeledi, do které náleží většina druhů, Curculionidae (Oberprieler et al. 2007, McKenna et al. 2009). Dle některých autorů je rozlišováno i více čeledí (Thompson 1992, Bouchard et al. 2011). Svojí početností jsou nosatcovití považováni za evolučně nejvyspělejší skupinu brouků (Crowson 1967). Skupina je celosvětově rozšířena a obývá většinu typů suchozemských a sladkovodních biotopů od arktické zóny po tropické lesy, kde diverzita dosahuje maxima (Oberprieler et al. 2007).

Nosatcovití jsou téměř výlučně herbivorní brouci často s monofágní nebo oligofágní vazbou především na krytosemenné rostliny (Magnoliophyta), méně často na nahosemenné (Pinophyta). Část druhů je saproxylických či fungivorních (Anthribidae, Cossoninae, Cryptorhynchinae, Scolytinae) s vazbou na odumírající dřevní hmotu nebo listový opad (Oberprieler et al. 2014). Odlišný vývoj mají větevníčci rodu *Anthribus*, respektive jejich larvy (Hoebeke & Wheeler 1991), kteří jsou predátoři puklicovitých (Coccidae) a mšicosavých (Sternorrhyncha). Larvy australských nosatců rodu *Tentegia* požírají klokaní trus (Wassel 1966) a brazilský nosatec *Ludovix fasciatus* je predátorem sarančích vajíček (Zwölfer & Bennett 1969). Některé druhy ze skupin Brentidae a Curculionidae jsou myrmekofilní (Marshall 1946, Sforzi & Bartolozzi 2004). U části druhů z čeledi Attelabidae je typický parazitismus, kdy dospělci obsazují již existující smotky jiných druhů zobonosek a kladou do nich vajíčka (Dieckmann 1974, Hamilton

1998). Někteří nosatcovití jsou také významnými opylovači cykasů, palem a primitivních krytosemenných rostlin a sehravají hlavní roli v přežití těchto ohrožených druhů (Oberprieler 2004).

Vývoj nosatcovitých probíhá v kořenech, stoncích, listech, trouchnivějícím dřevě, květech nebo v plodech. Vetší část druhů je oligofágních s vazbou na rod nebo čeleď rostlin, menší část tvoří monofágní a polyfágní druhy. Jednu živnou rostlinu dokáže obsadit několik druhů, např. na běžné pumpavě obecné (*Erodium cicutarium*) probíhá larvální vývoj čtyř druhů nosatcovitých – na květních pupenech se vyvíjí *Limobius borealis*, na listech *Brachypera dauci*, v kořeni *Lixus vilis* a v půdě v blízkosti řapíků listů *Coniocleonus nigrosuturatus* (Koch 1992, Příloha II). Vývoj jednotlivých druhů na jedné živné rostlině není oddělen pouze prostorově, ale v některých případech i časově do různých období roku (cf. Freude et al. 1981, 1983).

EKOLOGIE LARVÁLNÍCH STÁDIÍ NOSATCOVITÝCH

Nejběžnější typ larvy nosatcovitých brouků je slabě pigmentovaný a sklerotizovaný, bez končetin a tvaru písmene C. Larvy nosatcovitých žijí jak endofytním, tak endofágním způsobem života, ale koncepty endo/ektofytní a endo/ektofágní nejsou identické a je třeba je správně rozlišovat. Endofytní druhy žijí uvnitř rostlinných pletiv, kdežto označení endofágní odkazuje na žír uvnitř jakéhokoli substrátu (Oberprieler et al. 2014). Pouze několik taxonů má oba typy larev - jak endofytní, tak ektofytní, např. žír larev prvních instarů probíhá uvnitř stonků a později se larva přesouvá ze stonku do půdy, kde si tvoří hliněnou komůrku napojenou na stonek nebo kořen (Oberprieler et al. 2014).

Endofytní (a také endofágní) je většina larev nosatcovitých brouků, ale některé skupiny jsou i ektofytní. Ektofytní a také endofágní larvy typicky žijící v půdě mají např. Entiminae (12 000 druhů v 1 370 rodech), pro ně je typická strategie života larev mimo živnou rostlinu v blízkosti jejích kořenů, kterými se živí (Marvaldi et al. 2014). Ektofágní (a také ektofytní) jsou larvy žijící volně na exponovaných, vnějších částech rostlin, a jsou tak adaptovány k pohybu na rostlině. Pro pohyb slouží rudimentové hrudní výběžky nebo panožkovité výběžky, které nese posledních šest zadečkových článků (Oberprieler et al. 2014, Příloha V).

Pokusili jsme se zjistit nebo doplnit biologii a ekologii několika druhů, především v podčeledi Lixinae, které měly málo nebo naprosto neznámou bionomii a ve střední Evropě jsou vzácní nebo v ČR dokonce vyhynulí (cf. Stejskal et al. 2017). V první řadě se podařilo zjistit podrobnou ekologii druhu *Coniocleonus nigrosuturatus*, který žije pravděpodobně monofágně na pumpavě obecné (*Erodium cicutarium*), jeho larvy žijí endofytně mimo živnou rostlinu v hliněné komůrce, odkud probíhá žír stonku a řapíků. Tento způsob života je v rámci Lixinae ojedinělý a zatím ještě nebyl u žádného druhu pozorován (Příloha II). Dále se povedlo potvrdit již známé živné rostliny velmi vzácného druhu *Adosomus roridus* a zjistit, že larvy žijí endofytně v kořenovém krčku a v kořenech. A také podrobně popsat vývoj a ekologii tohoto druhu (Příloha III). V poslední fázi jsme se zaměřili na velmi vzácné druhy *Lixus bituberculatus* a *L. neglectus*, kteří mají společné, že žijí v malých areálech a jsou to ryze evropské druhy. Podařilo se nám zjistit detailní ekologii - u obou druhů jsou larvy endofytní a jsou to pravděpodobně monofágové nebo oligofágové. *Lixus bituberculatus* žije na čekance obecné (*Cichorium intybus*) a vývoj *L. neglectus* probíhá ve šťovíku rozvětveném (*Rumex thyrsiflorus*) (Příloha IV). Posledním studovaným taxonem byl *Eucoeliodes mirabilis* z podčeledi Ceutorhynchinae, u kterého jsme potvrdili už známou ekologii a navíc se podařilo objevit ektofytní larvy, které jsou v rámci nosatcovitých výjimečné, protože na svém těle nesou fekální štít, který je k tělu přichycen mikrotrichiemi. Tato naprosto jedinečná antipredační strategie je u nosatcovitých popsána zcela prvně a byla známa pouze u mandelinkovitých (Vencl & Morton 1999, Příloha V).

ZÁVĚR

Hlavní příčiny úbytku biodiverzity je nezbytné předem predikovat. Na pochopení těchto mechanizmů stojícími za snižováním diverzity je potřeba zvažovat mnoho faktorů (Fischer et al. 2003). Abychom mohli spolehlivě predikovat ohrožení hmyzu (Příloha I), je nutné co nejpřesněji znát bionomii jednotlivých druhů (Příloha II–V). Bez této znalosti jsou predikce neúplné a ochrana druhů je minimální, pokud neznáme jejich živné rostliny a přesné life history. Dosud se studie zaměřovaly na porovnání life history ohrožených i neohrožených druhů herbivorů směrem k propojení jejich klasifikace v červeném seznamu s jejich life history (cf. Kotiaho et al. 2005, Nolte et al. 2019). Mezi faktory, které ovlivňují riziko vyhynutí a ohroženost, by měly být zapojeny i další parametry prostředí, jako např. živné rostliny u herbivorních druhů. V rámci společenstev jsou herbivoři velmi významní a ovlivňují celý potravní systém (Polis & Winemiller 1996), což bylo zatím ve studiích zabývajících se predikcí ohroženosti převážně ignorováno.

V naší studii k predikci ohrožení herbivorních skupin hmyzu jsme si vybrali jako modelovou skupinu nosatcovité brouky (Curculionoidea), kteří jsou považovaní za indikátory zachovalosti prostředí (Benedikt et al. 2010) a jedná se zdaleka o největší skupinu herbivorů (Oberprieler et al. 2014). Predikci jsme testovali na základě life history nosatců a také ekologických vlastností jejich živných rostlin. Analýza odhalila, že největší riziko vyhynutí mají druhy monofágní, nelétavé, teplomilné, světlomilné, suchomilné a hygrofilní druhy s vazbou na otevřené biotopy. Obdobně je to i s vlastnostmi živných rostlin, které vysvětlují ohrožení nosatcovitých. Živné rostliny lze charakterizovat jako xerotermofilní a hygrofilní vyskytující se na biotopech s minimem dusíku. Nosatci, jejichž živné rostliny jsou obecně na ústupu a mají nízkou abundanci na stanovišti, jsou více náchylní k vyhynutí. A v neposlední řadě jsou ohroženější nosatci s vazbou na vodní rostliny a keříčkovitou vegetaci. Ze všech studovaných charakteristik se jako důležitější pro predikci ohrožení ukázalo life history nosatců než ekologické vlastnosti rostlin. Nicméně vlastnosti živných rostlin se zdají být důležité a měly by být použity pro predikční modely herbivorních skupin živočichů.

Dále jsme se zaměřili na odhalení biologie několika druhů, u kterých byla bionomie málo známá (Příloha III, V) nebo úplně neznámá (Příloha II, IV). U druhů *Adosomus roridus*, *Coniocleonus nigrosuturatus*, *Lixus bituberculatus* a *Lixus neglectus* se nám biologii a ekologii podařilo objasnit. V dalším případě jsme se zabývali detailní bionomií nosatce *Eucoeliodes mirabilis*, u kterého se kromě upřesnění biologie podařilo objevit i zajímavou adaptaci proti predátorům. Larva na svém těle nese tzv. fekální štít, který byl do té doby znám pouze u brouků z čeledi mandelinkovitých (Chrysomelidae).

Budoucí detailní výzkumy biologie a morfologie jednotlivých druhů mohou poskytnout zásadní informace pro efektivnější plánování pro biologické kontroly invazivních druhů rostlin. Výsledky významně přispívají i k základnímu výzkumu, ale budou mít i praktické důsledky pro ochranářskou biologii druhů a biologickou kontrolu.

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