Comparative Morphology of the Endophallus (Male Internal Genitalia) in Eight Species of the Genus *Lixus* Fabricius, 1801 (Coleoptera: Curculionidae: Lixinae): A Scanning Electron Microscope Study[1]

Mahmut Erbey[2] and Selami Candan[3]

Abstract: The fine structure of the endophallus in eight species of *Lixus* beetles [*L.* (*Callistolixus*) *furcatus* Olivier, 1807; *L.* (*Compsolixus*) *ascanii* (Linnaeus, 1767); *L.* (*Dilixellus*) *pulverulentus* (Scopoli, 1763); *L.* (*Epimeces*) *filiformis* (Fabricius, 1781); *L.* (*Epimeces*) *scolopax* Boheman, 1835; *L.* (*Lixochelus*) *cardui* Olivier, 1807; *L.* (*Lixoglyptus*) *circumcinctus* Boheman 1835; and *L.* (*Ortholixus*) *cinerascens* Schoenherr, 1832 (Coleoptera: Curculionidae: Lixinae)] was compared. The armatures of the endophallus (internal sac) were investigated with scanning electron microscopy. The surface morphology of the endophalli is different in all species investigated and these characters could be useful for species identification.

Key Words: Coleoptera, Curculionidae, *Lixus*; male genitalia, endophallus, scanning electron microscopy

Morphological structures of the male genitalia have been used widely for insect classification and identification (Sharp and Muir 1912, Snodgrass 1935, Lindroth 1957, Tuxen 1970, Lawrence et al. 2010, Medina et al. 2013). In weevils (Curculionidae), the external and internal male genitalia have important structures used to circumscribe higher taxonomic groups (Thompson 1988; Sert and Çağatay 1994, 1999; Sanmartín and Martín-Piera 2003; Velázquez de Castro et al. 2007; Wanat 2007; Bollino et al. 2017). Generally, the penis (or aedeagus) is tubular, sometimes flattened, often arcuate, and typically contains an internal, armed, eversible sac or endophallus (Tuxen 1970, Erbey et al. 2013, Schmidt and Uhl 2015). Typically, the eversible sac has an armature that intrudes into the lumen of the aedeagus when it is in repose. The endophallic membrane often has various sclerotised outgrowths forming variably shaped sclerites, miniplates, larger teeth, denticles, spines, spinules, scales, papillae, setae, etc. (Wanat 2007). The endophallus becomes everted when it inflates into the female vagina (or the bursa copulatrix) during coitus and is the functional intromittent organ. Upon eversion, the membranous surface of the internal sac often exposes the armature which is

---

[1] Received on February 19, 2018. Accepted on March 29, 2018. Last revisions received in July 31 2018.

[2] Ahi Evran University, Faculty of Arts and Science, Department of Biology, Kırşehir, Turkey. E-mail: merbey023@gmail.com. Corresponding Author.

[3] Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey. E-mail: scandan@gazi.edu.tr

DOI: 10.9784/LEB5(4)Erbey.01
Electronically available on August 3, 2018. Mailed on August 3, 2018.

suspected to affix the endophallus in the female genitalia after evagination [Figures 1 and 2; see also Schmidt and Uhl (2015) for an exceptional study on the reed beetles, *Donacia semicuprea* Panzer, 1796 and *Lilioceris lilii* (Scopoli, 1763) Chrysomelidae].

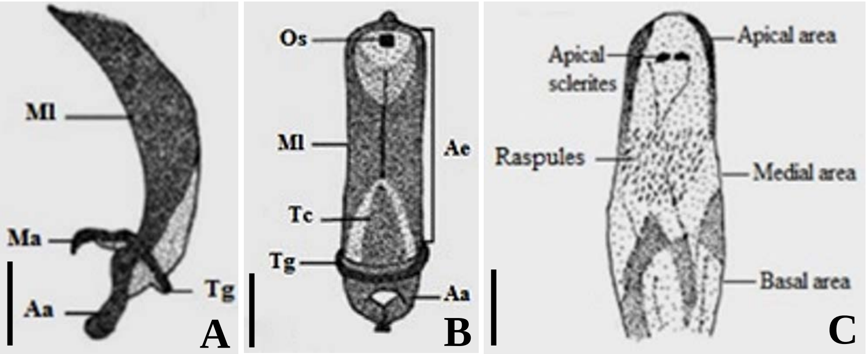

Figure 1. Aedeagus, or penis, of *Lixus cardui* Olivier, 1807. A. Lateral view. B. Dorsal view. C. Endophallus (ventral). Aa: Aedegal apodeme, Ae: Aedeagus, As: Apical sclerites, Ma: Manibrium, Ml: Median lobe, Os: Ostium, Tc: Aedegal tectum, Tg: Tegmen. Note, the authors consider the median lobe as a part of the penis or aedeagus. Scales for A and B = 0.5 mm; scale for C = 0.2 mm.

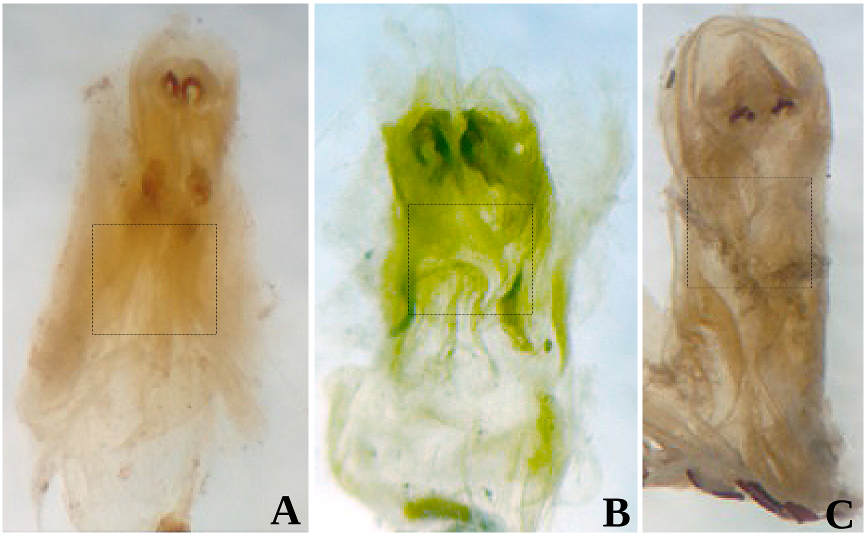

Figure 2. Light micrographs of the (ventral views) endophallus in three species of *Lixus*. A. (*Lixochelus*) *cardui*. B. *L*. (*Epimeces*) *scolopax*. C. *L*. (*Lixoglyptus*) *circumcinctus*. Raspules are located approximately in the middle of the endophallus (boxes).

The armature of the internal sac (Figure 2) appears to have a high degree of intraspecific stability and is has been useful to taxonomists (Sert and Çağatay

1994; Medina et al. 2013; Erbey and Candan 2015). Most likely because it is very difficult to dissect the endophallus out, especially in minute species, many researchers have used characters of the aedeagus in species descriptions but have not mentioned the endophallus (Angelov 1976; Caldara 1984, 1990; Dieckmann 1972, 1980; Erbey and Candan 2010, 2014, 2015; Hoffmann 1950, 1954; Pesarini 1980; Ter-Minasyan 1978; Yunakov and Korotyaev 2007). Increasing awareness of this structure and access to better imaging technology has increased our understanding of the endophallus' morphology. Sert and Çağatay (1994) described the endophallus of several species of *Sitona* (Curculionidae: Entiminae), *Bangasternus*, and *Larinus* (Curculionidae: Lixinae) and produced relatively simple illustrations. Sert and Çağatay (1999) investigated the endophallus in *Cleonus*, *Coniocleonus*, *Conorrhynchus*, *Larinus*, and *Lixus* (*L. cardui*, *L. circumcinctus*, and *L. filiformi*; Curculionidae: Lixinae), described the endophallus, and provided light micrographs of the apical sclerites (see also Erbey and Candan. 2015). Thereafter, Sert (2006) described the male genitalia of *Sitona fairmarie* Allard, 1869 including its endophallus. Medina et al. (2013) examined the external and internal male genitalia of 327 species of the dung beetles (Scarabaeidae: Scarabaeinae) and provided illustrations of the endophallus for 37 species. However, the exquisite morphological details of the endophallus, such as the raspules and the detailed shape of the teeth were not sufficiently revealed in Sert and Çağatay (1994) as well as in Medina et al. (2013) as compared with those available in this paper (e.g., Figures 4-7) using scanning electron microscopy.

Characteristics of the endophallus have been used for decades in ground beetles (Carabidae), especially in difficult groups (e.g., *Bembidion*; *Platypatrobus*, Lindroth 1961, 1962; *Sadonebria*, Sasakawa 2016; *Pterostichus*, Sasakawa and Itô 2018). The sclerotized structures of the endophallus have also been used to distinguish species of *Listronotus* (Curculionidae, Henderson 1940, O'Brien 1981).

Lixus Fabricius, 1801 is a genus of weevils (Curculionidae) with hundreds of named species. Importantly, several species of *Lixus* are noted agricultural pests. During his doctorate, author ME examined type specimens of all the species mentioned in this paper. The type material was useful to generate diagnostic museum materials with which to base subsequent species identifications. As far as the authors know, all names herein used are valid. Yet, not only are many of the species of *Lixus* similar externally but their aedeagi also look alike. Thus, the endophalus was explored as a possible structure to separate the species (Figures 3a- 3h). As far as we know, there is no published higher phylogeny of *Lixus* unto which the endophallic traits herein revealed could be mapped.

The aim of this study was to review the surface morphology of the endophallus in eight species of the weevil genus *Lixus* (Lixinae) using scanning electron microscopy (SEM). Herein, we reveal for the first time numerous characters of potential taxonomic value, such as spines, armature, teeth, and many others.

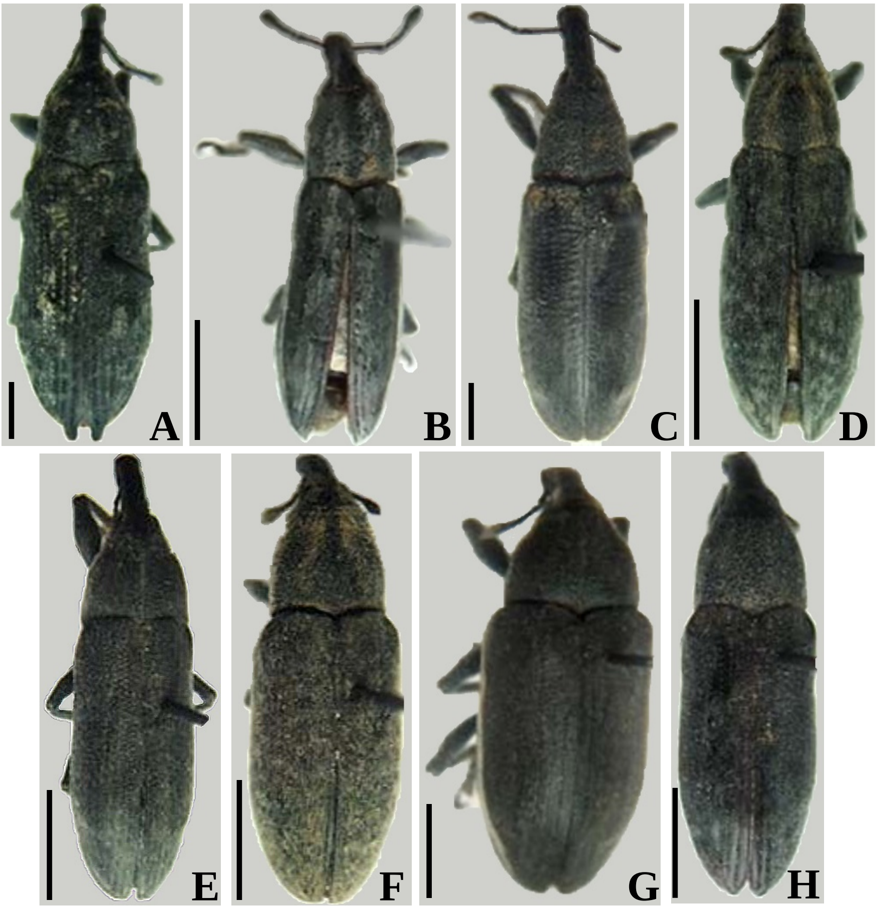

Figure 3. Dorsal view of a male from each of the eight species of *Lixus* studied. A. *L.* (*Callistolixus*) *furcatus* Olivier, 1807. B. *L.* (*Compsolixus*) *ascanii* (Linnaeus, 1767). C. *L.* (*Dilixellus*) *pulverulentus* (Scopoli, 1763). D. *L.* (*Epimeces*) *filiformis* (Fabricius, 1781). E. *L.* (*Epimeces*) *scolopax* Boheman, 1835. F. *L.* (*Lixochelus*) *cardui* Olivier, 1807. G. *L.* (*Lixoglyptus*) *circumcinctus* Boheman 1835. H. *L.* (*Ortholixus*) *cinerascens* Schoenherr, 1832. Scale bars for A, C, D, and H = 2 mm; B, E, F, and G = 3 mm.

Methods

Eight species of beetles belonging in the genus *Lixus* (Curculionidae: Lixinae) were investigated (organized alphabetically by subgenera): *Lixus* (*Callistolixus*) *furcatus* Olivier, 1807; *L.* (*Compsolixus*) *ascanii* (Linnaeus, 1767); *L.* (*Dilixellus*) *pulverulentus* (Scopoli, 1763); *L.* (*Epimeces*) *filiformis* (Fabricius, 1781); *L.* (*Epimeces*) *scolopax* Boheman, 1835; *L.* (*Lixochelus*) *cardui* Olivier, 1807; *L.* (*Lixoglyptus*) *circumcinctus* Boheman 1835; and *L.* (*Ortholixus*) *cinerascens*

Schoenherr, 1832. The specimens, which were originally collected in central Anatolia (Turkey), were selected from the Ahi Evran University Entomology Museum and and noted as vouchers with a label that reads "Endophallus studies Erbey and Candan". Classification of beetles follows Alonso-Zarazaga and Lyal (1999). Nomenclature for the endophallus follows Wanat (2007) and Medina et al. (2013).

For each species examined, three museum specimens were dissected with a light microscope and the aedeagus obtained after softening the abdomen in 10% KOH for 24 hours at 30ºC. The endophalli were then removed from the aedeagus and extended by fine dissection[4]. Observations and drawings were made on the display screen of the stereomicroscope (Olympus SZX12) at a magnification of 40x. For the scanning electron microscope study, three additional specimens were cleaned manually from organic debris and mounted on SEM specimen holders, or stubs, using double-sided sticky carbon tape, and subsequently coated with gold using a Polaron SC 502 Sputter Coater, and examined with a JOEL JSM 6060

[4] For a protocol to evert the inner sac, or endophallus, involving blowing air gently through the base of the median lobe of the aedeagus and its fascinating historical background, read Bontems (2013). A small portion of that work is herein translated, from French, as it reveals the very human, non-scientific aspects of science: "In 1975, Jacques Carayon, Director of the Laboratory of Entomology of the National Museum of Natural History in Paris, had entrusted to his assistant Nicole Berti, the mission to ensure the direction of my internship in Advanced Studies in Animal Biology, specialized in Entomology. For a few months, I became the student and thereafter during many years, a loyal collaborator of Nicole Berti, who had given me for subject a revision of the genus *Chrysochloa* = *Oreina* (Chrysomelidae) based mainly on the study of genitalia. Thus, through my internship, I became an expert in bibliographic research, knowledge of the collections and collectors of the [National Natural History] Museum and of Europe, [as well as the] methods of museology. By asking me not to disclose it (underscore added by authors), Nicole Berti also taught me a method of evagination of the inner membranous sac (endophallus) out of the aedeagus. I used to do this using her own equipment in her office when we were alone, but I had to remove it from the view of any visitor, whether [s/he] was a stranger to the Laboratory. This directive did not include Michel Rapilly, employee of Nicole Berti, a Cryptocephalinae specialist. His profession as a pharmacist led him to provide valuable advice on the use of laboratory products and materials, it would not be unlikely that he played a role in the development of the process of evagination of the inner sac, which he seemed to know. I never obtained from Nicole Berti the slightest precision on the inventor(s) of the process, which I attributed to her implicitly. As for her reasons for acting so discretely, it was obvious to me that she was preparing the publication of the process, constantly postponed by her concern with rigor and perfection. When I wrote my Master dissertation ... then published a part of my results ..., I could not therefore mention the method used, and found myself embarrassed to dodge the questions of fellow entomologists." Sasakawa and Itô (2018) reported inserting toothpaste into the aedeagus to inflate the endophallus using a 29-gauge needle. The male reproductive structures were preserved in ethanol and, after the toothpaste is inserted, let to dry. See also Thompson (1988) and van Dam (2014) for additional protocols.

SEM operated at low accelerating voltage, 5kV, to diminish beam damage to the fragile endophallic structures. Observations were made at 1,000x and 3,000x.

Results and Discussion

Using light microscopy, the endophallus of all examined specimens of *Lixus* is a transluscent membrane with many folds and its surface is strongly covered with spots. The endophallus has two apical sclerites located dorsomedially. Armature structures, such as teeth, spines, denticles (or raspules, as of Medina et al. 2013) are located in the dorsomedial area of the endophallus; they are also located on the ventral surface (Figure 1C). The term raspule (singular, raspula; plural, raspulae or raspules) has been used in the literature of the endophallus in the Scarabaeidae. Herein, we used the term, raspule, to mean an area of sculpturation and/or setation on the endophallus. A distinct element within a raspule, as observed with the scanning electron microscope, is called tooth, microscale, seta, etc. depending on its overall shape.

In contrast, scanning electron microscopy reveals a host of strongly sclerotized structures in exquisite detail, particularly on the ventral surface. For example, the endophallic rapsules have teeth, spines, and denticles. Those structures are different in all species examined. The raspules are strongly covered with teeth that form two or three groups that join basally (*Lixus pulverulentus*, Figures 5A-C); the teeth are very short and join forming ranges (*L. ascanii*, Figures 4D-F); raspules have folds each with long teeth (*L. cardui*, Figures 6D-F); the teeth are short and forming regular groups (*L. circumcinctus*, Figures 7A-C); the surface has strong teeth that generally form three groups, the middle tooth is much longer than the others (*L. cineracens*, Figures 7D-F); the teeth are well-developed, short, and appear like fringes (*L. filiformi*, Figures 5D-F); the surface have teeth that are long and form groups of two or three (*L. furcatus*, Figures 4A-C); or the teeth are strong and form short, flat groups (*L. scolopax*, Figures 6A-C). These characteristics could be useful to generate a higher classifcation of *Lixus* and may help to distinguish similar-looking species based on morphological traits of the endophali, especially if the aedeagi are also similar.

Numerous important questions remain to be answered. For example, how does the environment may affect chitin deposition in the endophallus and alter the phenotype of individuals? How the shape and other morphological features of the endophallus may relate to the shape of female genitalia, their role in sexual selection, and speciation (Eberhard 1985, Masly 2012, Hosken and Stockley 2004, Méndez and Córdoba-Aguilar 2004)? Does *Lixus* have dimorphic aedegi, where the aedeagus is “right-handed” or “left-handed” when protruded for insertion into the female, as it has been found in other beetles? (Liebherr and Will 2015).

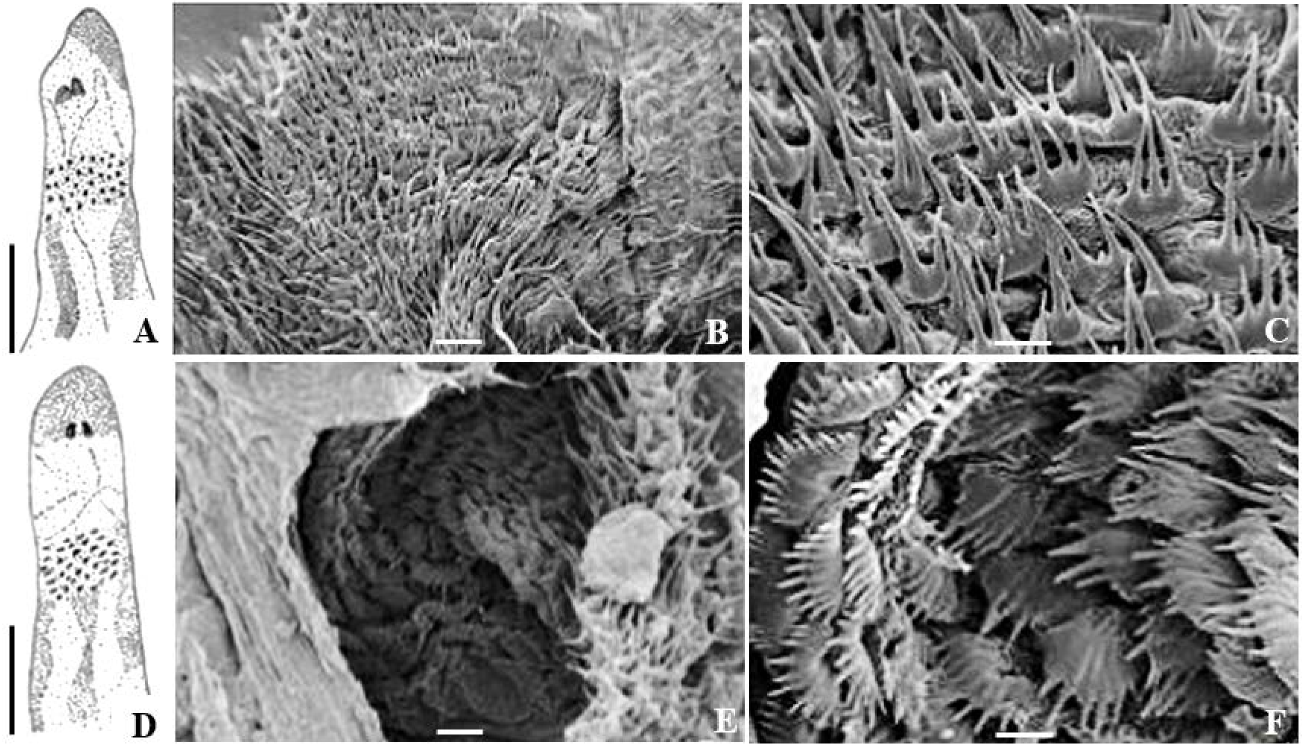

Figure 4. A-F. Endophalli of *Lixus furcatus* and *L. ascanii*. A.-C. *Lixus* (*Callistolixus*) *furcatus* Olivier, 1807. D.-F. *Lixus* (*Compsolixus*) *ascanii* (Linnaeus, 1767). A. and D. Drawing at low magnification using a light microscope. Scale bars = 0.3 mm, 0.2 mm, respectively. B. and E. Scanning electron micrographs taken at 1000x, scale bars = 10 µ. C. and F. Scanning electron micrographs taken at 3,000x, scale bars = 5 µ.

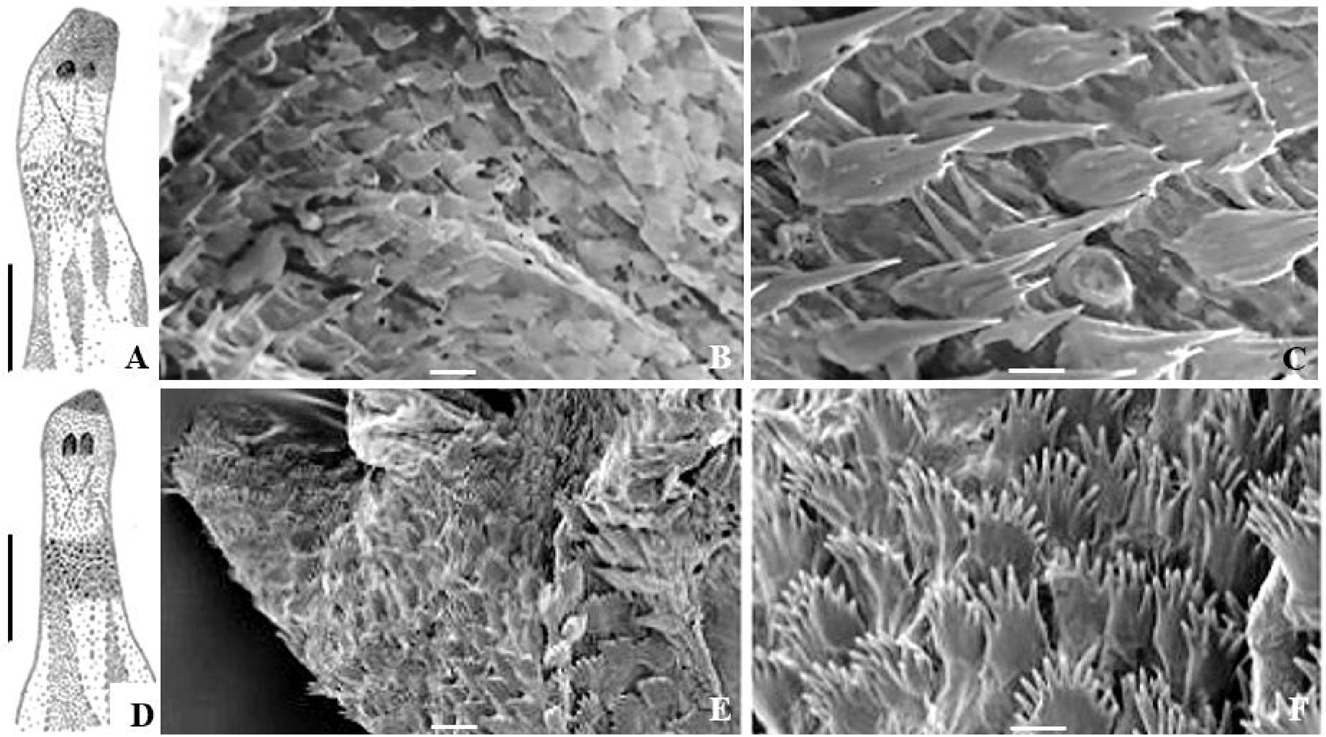

Figure 5. A-F. Endophalli of *Lixus pulverulentus* and *L. filiformis*. A.-C. *Lixus* (*Dilixellus*) *pulverulentus* (Scopoli, 1763). D.-F. *Lixus* (*Epimeces*) *filiformis* (Fabricius, 1781). A. and D. Drawing at low magnification using a light microscope. Scale bars = 0.3 mm, 0.2 mm, respectively. B. and E. Scanning electron micrographs taken at 1000x, scale bars = 10 µ. C. and F. Scanning electron micrographs taken at 3,000x, scale bars = 5 µ.

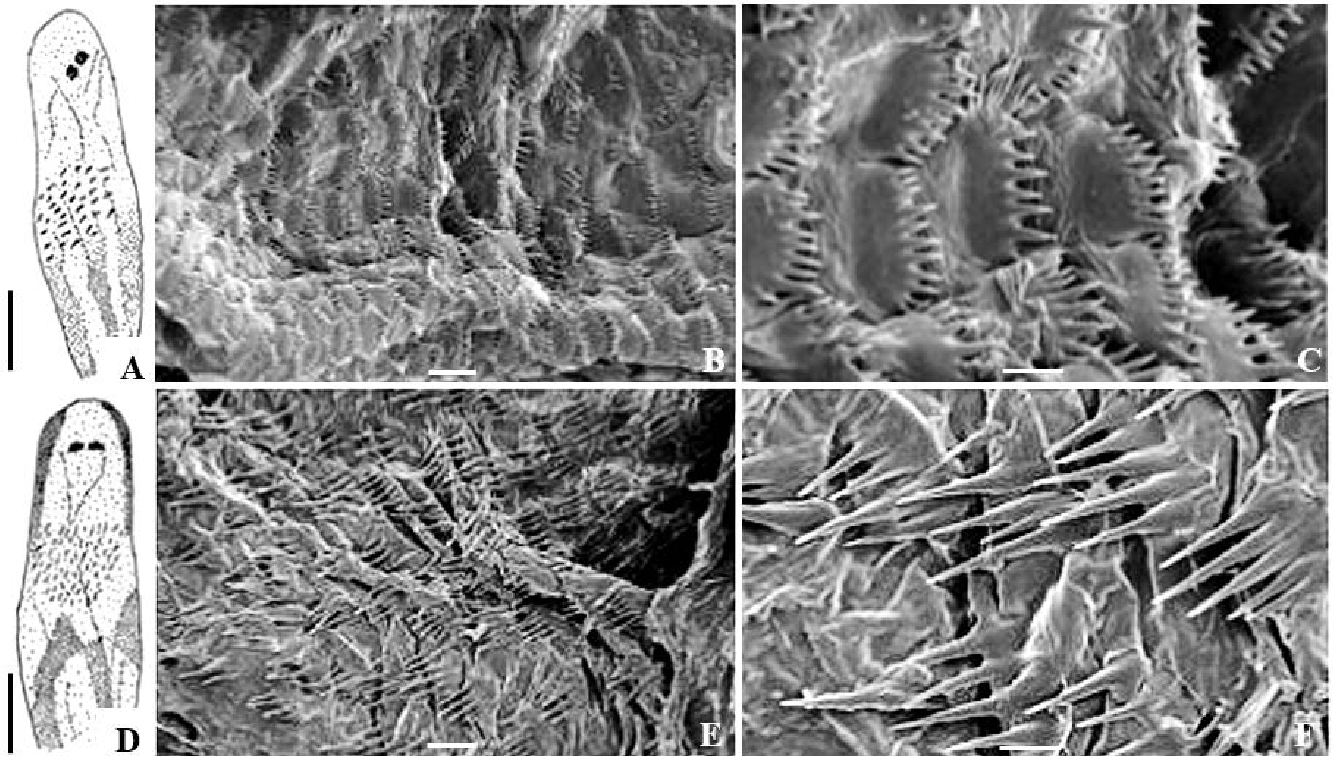

Figure 6. A-F. Endophalli of *Lixus scolopax* and *L. cardui*. A.-C. *Lixus* (*Epimeces*) *scolopax* Boheman, 1835. D.-F. *Lixus* (*Lixochelus*) *cardui* Olivier, 1807. A. and D. Drawing at low magnification using a light microscope. Scale bars = 0.3 mm, 0.2 mm, respectively. B. and E. Scanning electron micrographs taken at 1000x, scale bars = 10 µ. C. and F. Scanning electron micrographs taken at 3,000x, scale bars = 5 µ.

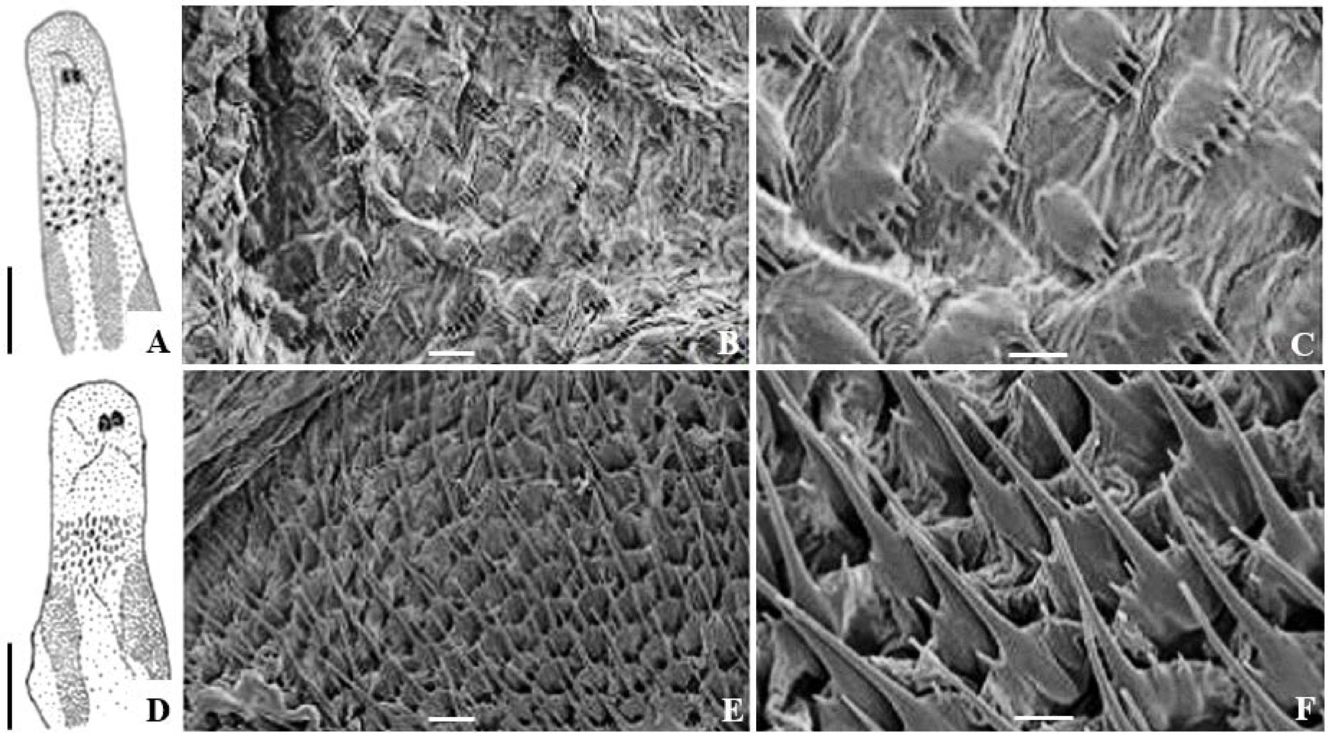

Figure 7. A-F. Endophalli of *Lixus circumcinctus* and *L. cinerascens*. A.-C. *Lixus* (*Lixoglyptus*) *circumcinctus* Boheman 1835. D.-F. *Lixus* (*Ortholixus*) *cinerascens* Schoenherr, 1832. A. and D. Drawing at low magnification using a light microscope. Scale bars = 0.2 mm. B. and E. Scanning electron micrographs taken at 1000x, scale bars = 10 µ. C. and F. Scanning electron micrographs taken at 3,000x, scale bars = 5 u.

Acknowledgements

We thank the Project Direction Office of the Ahi Evran University, Merkez/Kırşehir in Turkey for supporting this project (Project no: PYO-FEN.4001.14.012). Dr. James Bergdahl (Conservation Biology Center, Spokane, Washington, USA), David H. Kavanaugh (Department of Entomology, California Academy of Sciences, San Francisco, California, USA), and Dr. Terry Erwin (Department of Entomology, National Museum of Natural History, Washington, District of Columbia, USA) directed us to important papers on the reproductive structures of male Carabidae. Dr. Koji Sasakawa (Laboratory of Zoology, Department of Science Education, Chiba University, Chiba, Japan) and Dr. Christian Bontems (Musée' d'Histoire Naturelle, Paris) provided additional details on eversion techniques of the internal sac. Mr. Guy Hanley (Northern Plains Entomology, Willow City, North Dakota, USA) directed us to pertinent literature about the endophallus in Curculionidae. A colleague who prefers to remain anonymous helped decipher the probable meaning of "Rekv. nr 35" placed on the cover page of Lindroth 1961. Andrew Bachman (The Pennsylvania State University, York, Pennsylvania, USA) greatly assisted the editor with Photoshop. Eight reviewers offered constructive suggestions to this paper.

Literature Cited

Alonso-Zarazaga, M. A. and C. H. C. Lyal. 1999. A World Catalogue of Families and Genera of Curculionoidea (Insecta: Coleoptera) (Excepting Scolytidae and Platypodidae). *Entomopraxis* pp. 1-315. http://weevil.info/sites/weevil.info/files/Alonso-Zarazaga%20%26%20Lyal,%201999_World%20Catalogue%20(searchable).pdf

Angelov, P. 1976. *Fauna Bulgarica. 5, Coleoptera, Curculionidae, Part I* (*Apioninae, Otiorhynchinae*). Sofya, Bulgaria. 356 pp.

Bollino, M., F. Sandel, and A. Rukmane. 2017. New species of the genus Pachyrhynchus Germar, 1824 (Coleoptera: Curculionidae) from Mindanao, Philippines. *Baltic Journal of Coleopterology* 17(2):189-204.

Bontems, C. 2013. Le procédé Berti-Vachon d'évagination du sac interne. *Nouvelle Revue d'Entomologie* (*Nouvelle Série*) 29(1):85-91.

Caldara, R. 1984. Revision della *Sibinia* palearctiche (Coleoptera, Curculionidae). *Memorial Society Entomological Italia* 63:24-105.

Caldara, R. 1990. Revision taxonomica della specie paleartiche del genere *Tychius* Germar (Coleoptera: Curculionidae). *Memorie Society Italia Science National Museum Civil Storie Nature* 25:45-217.

Dieckmann, L. 1972. Beitrage zur insektenfauna der DDR: Coleoptera-Curculionidae: Ceutorhynchinae. *Beitrage Entomologica* 22:3-128.

Dieckmann, L. 1980. Beitrage zur insektenfauna der DDR: Coleoptera-Curculionidae (Brachycerinae, Otiorrhynchinae, Brachyderinae). *Beitrage Entomologica* 30:145-310.

Eberhard, W. G. 1985. *Sexual Selection and Animal Genitalia*. Harvard University Press. Cambridge, Massachusetts, USA. 244 pp. https://doi.org/10.4159/harvard.9780674330702

Erbey, M. and S. Candan. 2010. Description of surface morphology in male and female genitals of *Lixus cardui* Olivier, 1807 (Coleoptera: Curculionidae: Lixinae) a scanning electron microscope study. *Acta Zoologica Bulgarica* 62:321-329. http://www.acta-zoologica-bulgarica.eu/downloads/acta-zoologica-bulgarica/2010/62-3-321-330.pdf

Erbey, M., S. Candan, and E. Koçak. 2013. The male genitalia in *Lixus nordmanni* Hochhuth, 1847 (Coleoptera: Curculionidae: Lixinae); studing by light and scanning electron microscope. *Gazi University. Journal of Science* 26(4): 509-513. http://gujs.gazi.edu.tr/article/view/1060000776/1060000793

Erbey, M. and S. Candan. 2014. Ultrastructural analysis of the internal sac in several species of the weevil tribe Phyllobiini (Coleoptera: Curculionidae: Entiminae). *Zoology in the Middle East* 60(4):339-344. https://doi.org/10.1080/13546805.2014.970369

Erbey, M. and S. Candan. 2015. Ultrastructural analysis of endophallus in several species of the genus *Larinus* Dejean, 1821 (Coleoptera: Curculionidae: Lixinae). *Journal of Entomology and Zoology Studies* 3(2):54-56.

Henderson, L. S. 1940. A revision of the genus *Listronotus*. University of Kansas Science Bulletin 26:215-337 + illustrations.

Hoffmann, A. 1950. Faune de France 52, Coleopteres, Curculionides. Premiere Partie. Paris, France. pp. 1-486.

Hoffmann, A. 1954. Faune de France 59, Coleopteres, Curculionides. Deuxieme Partie. Paris, France. pp. 487-1208.

Hosken, D. J. and P. Stockley. 2004. Sexual selection and genital evolution. *Trends in Ecology and Evolution* 19:87–93. https://doi.org/10.1016/j.tree.2003.11.012

Lawrence, J. F., R. G. Beutel, R. A. B. Leschen, and S. A. Ślipiński. 2010. Glossary of morphological terms. pp. 9-20. *In*, Leschen, R. A. B., R. G. Beutel, and J. F. Lawrence (Volume Editors). *Handbook of Zoology*. Arthropoda: Insecta. Coleoptera, Beetles. Volume 2: Morphology and Systematics (Elateroidea, Bostrichiformia, Cucujiformia, partim). Walter de Gruyter GmbH & Co. KG. Berlin, Germany. 785 pp.

Liebherr, J. K., and K. W. Will 2015. Antisymmetric male genitalia in Western Australian populations of *Mecyclothorax punctipennis* (Coleoptera: Carabidae: Moriomorphini). *Insect Systematics & Evolution* 46:393-409. https://doi.org/10.1163/1876312X-45042124

Lindroth, C. H. 1957. The principal terms used for male and female genitalia in Coleoptera. *Opuscula Entomologica* 22:241-256.

Lindroth, C. H. 1961. Skalbaggar. Coleoptera. Sandjägare och Jordlöpare. Fam. Carabidae. *Svensk Insektfauna* 9. Utgiven av Entomologiska Föreningen i Stockholm. Stockholm, Sweden. 209 pp. [The phrase, “Rekv. nr 35”, which is printed in the upper right hand corner of the cover page of this work, appears to mean “rekvisition" or "order", thus requisition number in Danish, https://mymemory.translated.net/en/Danish/English/rekv-nr .]

Lindroth, C. H. 1962. The male of *Platypatrobus lacustris* Darlington (Coleoptera: Carabidae). *Psyche* 69(1)7-10. https://doi.org/10.1155/1962/85681

Masly, J. P. 2012. 170 Years of “Lock-and-Key”: Genital morphology and reproductive isolation. *International Journal of Evolutionary Biology* Article ID 247352, 10 pp. http://dx.doi.org/10.1155/2012/247352

Méndez, V. and A. Córdoba-Aguilar. 2004. Sexual selection and animal genitalia. *Trends in Ecology and Evolution* 19(5):224–225. https://doi.org/10.1016/j.tree.2004.03.012

Medina, C. A., F. Molano, and C. H. Scholtz. 2013. Morphology and terminology of dung beetles (Coleoptera: Scarabaeidae: Scarabaeinae) male genitalia. *Italia Entomologica* 8:239-250. https://doi.org/10.11646/zootaxa.3626.4.3

O’Brien, C. 1981. The larger (4.5+ mm.) *Listronotus* of America, noth of Mexico (Cylindrorhininae, Curculionidae, Coleoptera). *Transactions of the American Entomological Society* 107:69-123. http://www.jstor.org.ezaccess.libraries.psu.edu/stable/pdf/25078279.pdf?refreqid=excelsior%3Aa0b211afcb383638f9f323a867f34c21

Pesarini, C. 1980. Le specie paleartiche occidentalli della tribü Phyllobiini (Coleopera: Curculionidae). *Bolletino de Zoología Agraria e Bachicultura* 2:49-230.

Sasakawa, K. 2016. Two new species of the ground beetle subgenus *Sadonebria* Ledoux & Roux, 2005 (Coleoptera, Carabidae, Nebria) from Japan and first description of larvae of the subgenus Zookeys 578:97-113. https://doi.org/10.3897/zookeys.578.7424; https://zookeys.pensoft.net/articles.php?id=7424

Sasakawa, K. and H. Itô. 2018. Two new species of the *Pterostichus macrogenys* species group (Coleoptera, Carabidae) discovered in shallow subterranean habitats in northern Honshu, Japan. Subterranean Biology 21:47-46. https://doi.org/10.3897/subtbiol.21.11155

Sanmartín, I. and F. Martín-Piera. 2003. First phylogenetic analysis of the subfamily Pachydeminae (Coleoptera, Scarabaeoidea, Melolonthidae): the Palearctic Pachydeminae. *Journal of Zoological Systematics and Evolutionary Research* 41:2-46. https://doi.org/10.1046/j.1439-0469.2003.00179.x

Schmidt, M. and G. Uhl. 2015. Functional morphology of the copulatory organs of a reed beetle and a shining leaf beetle (Coleoptera: Chrysomelidae: Donaciinae, Criocerinae) using X-ray micro-computed tomography. *Zookeys* 547:193-203. https://doi.org/10.3897/zookeys.547.7143

Sert, O. 2006. On the female and male genital structures of *Sitona fairmairei* Allard, 1869 (Coleoptera: Curculionidae) from Turkey. *Entomological News* 117:441- 443. https://doi.org/10.3157/0013-872X(2006)117[441:OTFAMG]2.0.CO;2

Sert, O. and N. Çağatay. 1994. Systematic studies on some species of genera *Sitona*, *Bangasternus* and *Larinus* (Coleoptera: Curculionidae) (in Turkish). *Turkish Journal of Entomology* 18:223-236.

Sert, O. and N. Çağatay. 1999. Taxonomic studies on some species of the subfamily Cleoninae (Coleoptera: Curculionidae) from central Anatolia (in Turkish). *Turkish Journal of Entomology* 23:817-827.

Sharp, D. and F. Muir. 1912. XI. The comparative anatomy of the male genital tube in Coleoptera. *Transactions of the Entomological Society of London* Part III:477-642 + plates. https://www.biodiversitylibrary.org/item/43638#page/3/mode/1up

Snodgrass, R. E., 1935. *Principles of Insect Morphology*. McGraw-Hill Book Company. New York, NY, USA. 646 pp.

Ter-Minasyan, M. E. 1978. Weevils of the subfamily Cleoninae in the fauna of the USSR. Tribe Lixini (in Russian). Zoological Instutite Academy of Sciences of the USSR, 1-166.

Thompson, R.T. 1988. Revision of the weevil genus *Leptostethus* Waterhouse, 1853 (Coleoptera: Curculionidae, Entiminae). *Cimbebasia Memoir* 7:1-80.

Tuxen, S. L. 1970. *Taxonomist's Glossary of Genitalia in Insects*. E. Munksgaard. Copenhagen, Denmark. 215 pp.

Van Dam, M. H. 2014. A simple, rapid technique for the inflation of the endophallus, with particular focus on the Curculionoidea (Coleoptera). *The Coleopterists Bulletin* 68(2):263-268.

Wanat, M. 2007. Alignment and homology of male terminalia in Curculionoidea and other Coleoptera. *Invertebral Systematics* 21: 147-171. https://doi.org/10.1071/IS05055

Yunakov, N. N. and B. A. Korotyaev. 2007. Review of the weevil subgenus *Metaphyllobius* Smirnov (Coleoptera, Curculionidae, Entiminae) from Eastern Europe and Siberia. *Entomological Review* 87:1045–105. https://doi.org/10.1134/S0013873807080106