



**Two sibling species of *Leiopus* Audinet-Serville, 1835
(Coleoptera: Cerambycidae) from Europe:
L. nebulosus (Linnaeus, 1758) and *L. linnei* sp. nov.**

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Abstract

The common European cerambycid *Leiopus nebulosus* (Linnaeus, 1758) is herein split into two sibling species. The male genitalia characters, as well as spermathecae in females, were examined and found to provide strong support for this separation. A new species, *Leiopus linnei* sp. nov., is based on specimens mainly from Scandinavia. The establishment of the new species is supported by DNA barcoding of Scandinavian specimens of *L. nebulosus*, *L. linnei* sp. nov., and *L. punctulatus* (Paykull, 1800). There are significant genetic differences between all these species. The geographical distribution and the bionomy of *L. nebulosus* and *L. linnei* sp. nov. are described. The type of *Cerambyx nebulosus* Linnaeus, 1758 is lost. A neotype of *Cerambyx nebulosus*, currently *Leiopus nebulosus* (Linnaeus, 1758), is designated and a redescription of *L. nebulosus* is presented. A key for the identification of *L. nebulosus* and *L. linnei* sp. nov. is provided. The varieties *L. nebulosus* var. *dissimilis* Pic, 1889, *L. nebulosus* var. *unifasciatus* Pic, 1891, and *L. nebulosus* var. *siculus* Pic, 1924 are considered as junior synonyms, **syn.nov.** of *L. nebulosus*.

Key words: Acanthocinini, *Leiopus linnei* sp. nov., sibling species, *Leiopus nebulosus* (Linnaeus, 1758), *Leiopus punctulatus* (Paykull, 1800), Palearctic Region, DNA, barcoding

Introduction

The cerambycids of Northern Europe are among the most thoroughly studied insects (c.f. Sama 2002; Ehnström & Holmer 2007). However, there are still a number of unanswered taxonomical questions regarding the family in Northern Europe. We believe that some of these questions can only be answered by more thorough studies of the genitalia, and use of DNA-analysis. We also believe that such studies will uncover new sibling or cryptic species. Once discovered and thoroughly studied, the latter, according to Mayr (1969), are usually found to have previously overlooked morphological differences. The sibling species concept requires that the genetic divergence is evident, and that they occur sympatrically (Mayr 1979).

The genus *Leiopus* was erected by Audinet Serville (1835), although *L. nebulosus* (Linnaeus, 1758) was first designated as the type species of the genus by Thomson (1866). *Leiopus nebulosus* (Linnaeus, 1758) is known to be a highly variable species with respect to external morphological characters such as coloration, punctuation and size (Bense 1995; Cherepanov 1991; Freude *et al.* 1966; Sama 2002; Villiers 1978; Vives 2000). It is also known to be a highly polyphagous species exploiting a wide range of host trees, including both deciduous and coniferous trees (Demelt 1966; Bense 1995; Sama 2002; Heliövaara *et al.* 2004). It has an extensive geographical distribution (Sama 2002), with geographical variations and morphological differences between populations. The hypothesis that the species "apparently has failed, during the process of speciation,

to acquire conspicuous morphological differences" (*sensu* Mayr 1979), was tested.

We have examined *L. nebulosus* from a large number of localities (varying between island, coastal, and inland habitats), mainly from Scandinavia, but also from the British Isles, central and southern Europe including the Mediterranean area. We used a combination of characters, including the male genitalia and female spermathecae, to separate *L. nebulosus* into two sibling species. The morphological results were supported by DNA-analyses. We have also consulted the old literature because there are some nomenclatural problems in *L. nebulosus*.

Material and methods

A total of 300 European specimens (174 males and 126 females) of the two sibling species, previously known as *Leiopus nebulosus*, were examined, of which a total of 146 males and 36 females were dissected. The genitalia characters of the two sibling species of *Leiopus* were compared with other Palaearctic species of *Leiopus*. A DNA-analysis was undertaken to investigate any genetic difference between the two sibling species of *Leiopus*. We have also examined the available historical types, retained in museum collections, to establish the identity of the names included in original descriptions and/or cited in the old literature.

The morphological characters we used in distinguishing the new sibling species from *L. nebulosus* are differences in the male genitalia, and the sclerotized part of the female spermatheca. The male genitalia characters have recently been used successfully to separate closely related species of European Lamiinae in the genera *Leiopus* Audinet Serville, 1835 (Sama 1985) and *Stenostola* Mulsant, 1839 (Wallin, Lundberg & Hägg 2005). We also compared the width of frons in the two sibling species of *Leiopus*, with measurements including the greatest distance between the lowest parts of the eyes that was measured on 20 specimens of each species (10 males/10 females). Specimens from a total of 25 different localities were included in the analysis.

Detailed information on the distribution of records from Scandinavia is outlined in Fig. 20. The examined specimens were collected during mid-May to mid-August, and were either sampled from dead tree branches in the field, or reared from the host tree substrates.

List of abbreviations

| | |
|-------|--|
| BMNH | Natural History Museum, London |
| GNM | Göteborg Natural History Museum, Gothenburg |
| MNHNP | Muséum National d'Histoire Naturelle, Paris |
| NHMO | Natural History Museum, Oslo, which contains the collections from the formerly Norwegian Forest Research Institute, the Norwegian Plant protection Institute and the collection of Torstein Kvamme, Ås |
| NRM | The Swedish Museum of Natural History, Stockholm |
| SLU | Swedish University of Agricultural Sciences, Dept. of Ecology, Uppsala |
| UUZM | Museum of Evolution - Zoology, Uppsala University: formerly Uppsala University, Zoological Museum, Uppsala |
| ZMH | Zoological Museum, Finnish Museum of Natural History, University of Helsinki, Helsinki |
| ZMUC | Zoological Museum, Natural History Museum of Denmark, Copenhagen University, Copenhagen |
| ZML | Zoological Museum, Lund University, Lund |

Abbreviations of private collections are listed below:

| | |
|----|---|
| AB | Allesandro B. Biscaccianti, Italy, Rome |
| AD | Alan Dufberg, Sweden, Malmö |
| BA | Bengt Andersson, Sweden, Nybro |

| | |
|-----|--|
| BE | Bengt Ehnström, Sweden, Nås |
| CH | Carolus Holzschuh, Austria, Villach |
| GG | Gösta Gillerfors, Sweden, Varberg |
| HW | Henrik Wallin, Sweden, Uppsala |
| KS | Kristoffer Stighäll (ex. coll. Rune Axelsson), Sweden, Norrtälje |
| MS | Milan Slama, Czech Republic, Prague |
| ON | Ove Nodmar, Sweden, Holmsjö |
| PB | Pierre Berger (ex. coll. Milan Slama), France, Meylan |
| RP | Roger Pettersson, Sweden, Umeå |
| SL | Stig Lundberg, Sweden, Luleå |
| SvL | Sven Lennartsson, Sweden, Mjölby |
| SM | Sture Marklund, Sweden, Örebro |
| SO | Stefan Olberg, Norway, Bärum |
| TK | Torstein Kvamme, Norway, Ås |
| UN | Ulf Nylander, Sweden, Gävle |
| ÅL | Åke Lindelöw, Sweden, Uppsala |

Examined material

Leiopus nebulosus: **Sweden** (49 males, 34 females, NRM, ZML, SLU, AD, BE, GG, HW, KS, ON, SL, SM, SvL, UN, ÅL); **Norway** (7 males/2 females, NHMO). **Denmark** (7 males, 9 females, ZMUC). **Finland** (1 male, 4 females, ZMH). **France** (2 males, 1 female, HW). **Germany** (1 female, ZML). **British Isles** (4 males, 4 females, BMNH). **Ireland** (2 females, BMNH). **Italy** (13 males/5 females, AB, ÅL). **Thunberg** (1 male), **Paykull** (1 female), **Boheman** (3 males/2 females), **Fabricius** (no specimens retained), **Banks** (several unlabelled specimens retained, S. Shute per comm.).

Leiopus linnei **sp.nov.**: **Sweden** (43 males, 34 females, GNM, NRM, SLU, UUZM, ZML, AD, BA, GG, HW, KS, ON, SL, UN). **Norway** (8 males, 1 female, NHMO, SO). **Denmark** (14 males, 10 females, ZMUC, ZML). **Germany** (2 males, 2 females, ZML). **Poland** (1 female, BA). **France** (1 female, MS). **Czech Republic** (3 males, BA, MS). **Slovakia** (1 female, MS). **Austria** (1 male, 1 female, MS). **Bulgaria** (5 males, 1 female, ZML, ÅL). **Rumania** (1 male, ZML). **Croatia** (1 male, ZML). **British Isles** (2 males, 4 females, BMNH). **Thunberg** (2 males, 1 female). **Paykull** (3 males, 1 female). **Gyllenhal** (2 males, 3 females).

The following additional Palaearctic species of *Leiopus* were examined: *L. nebulosus* ssp. *caucasicus* Ganglbauer (Russia, Caucasus, 1 male, 2 females, SL, HW). *L. punctulatus* (Paykull) (5 males, 1 female), *L. albivittis* (Kraatz) (Russia, 2 males, SL, HW), *L. insulanus* Slama (Greece, Crete, 1 male, PB), *L. bedeli* Pic (Algeria, 1 female, SL), *L. kharazii* Holzschuh (Iran, 2 males, 2 females, CH, UN), *L. femoratus* Fairmaire (France, Russia, Turkey, 7 males, 1 female, HW, SL), *L. syriacus* Ganglbauer (Turkey, 2 males, 2 females, RP, UN), *L. montanus* Hayashi (Japan, 1 male, 1 female, UN), *L. stillatus* (Bates) (Russia, Japan, 2 males, 1 female, UN), and *L. guttatus* Bates (Japan, 1 male, 1 female, UN).

Genitalia characters

The sclerotized parts of the male genitalia including the aedeagus, the sclerites inside the internal sac attached to the aedeagus, parameres and proctiger (the 8th tergum) were used to separate the two sibling species. The parameres, or lateral lobe, in *Leiopus* spp. are slender, long and flattened dorso-ventrally with an apical fringe of short hairs and with an extended base of tegmen. We also examined the sclerotized part of the spermatheca in females, which is described for the first time in the genus *Leiopus*. The method is outlined in detail in previous publications (Wallin, Lundberg & Hägg 2005; Wallin & Nylander 2006, 2007). These microscopic characters appear to be species-specific and have been described for the following species of *Leiopus*: *L. nebulosus*, *L. punctulatus*, *L. bedeli*, *L. syriacus*, *L. settei* (Sama, 1985), *L. andreae* (Sama, 1994), and *L. femoratus* (Biscaccianti 2005).

DNA-analysis

Full-grown larvae, freshly collected from branches at the same localities as specimens included in the morphological studies, were used for DNA-extraction. The larvae were preserved in absolute alcohol. Larvae of *L. linnei* **sp. nov.** were collected from *Quercus robur* during May 2007 at Biskops-Arnö, Uppland in Sweden. *L. nebulosus* larvae were collected from *Corylus avellana* at Strandtorp, the island of Öland, Sweden, during May 2007. The third Scandinavian species, *L. punctulatus*, was included for comparison, and one larva from Björkö in the province of Uppland, Sweden was collected from *Populus tremula* in May 2007. DNA extraction was undertaken according to the instructions of the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek). PCR amplification of the “barcode” region of CO1 followed the standard procedure described by Hebert et al. (2004). The primer used in this study was the standard barcoding primer; FWD TTCTCCAACCACAAAGACATTGGCAC and REW ACGTGGGAGATAATTCCAAATCCTG. The longhorn species *Tetraopes texanus* Horn (Cerambycidae, Lamiinae, Tetraopini) was used as outgroup for comparison (GenBank no. AF267475). A neighbour-joining tree was constructed, using p-distances, with 1000 bootstrap replicates.

Taxonomy

Leiopus nebulosus (Linnaeus, 1758)

Cerambyx nebulosus Linnaeus, 1758: 627. The type is lost.

Designation of a neotype for *Cerambyx nebulosus*: A neotype (male with body length: 7.6 mm, width: 2.3 mm): Sweden, Gotland, Sproge, 1972-06-19, leg. Bengt Ehnström, was designated from the examined material and deposited in the type collection at UUZM with identity= UUZM Types No. 2823. The neotype is collected relatively near the type locality mentioned by Linnaeus (1745, 1758). The designation of a neotype is made due to loss of the original type of *Cerambyx nebulosus* Linnaeus, 1758. Thunberg (1787) was unable to separate *Cerambyx nebulosus* from the new sibling species, and none of the preserved material is supplied with labelling data, or insect pins that could be identified as clearly originating from the Linnean collection. We have therefore designated a more recently collected specimen as the neotype of *Cerambyx nebulosus*.

Synonyms: *Cerambyx bifasciatus* Goeze, 1777: 464 (nec Linnaeus, 1767 according to Vives, 2000)

= *Cerambyx taeniatus* Gmelin, 1790: 1863

Cerambyx niger Geoffroy, 1762: 204

Cerambyx monilis Geoffroy, 1785: 75

Cerambyx fasciatus Villers, 1789 : 239 (nec Scopoli, 1763, nec DeGeer, 1775, nec Fabricius, 1775, nec Geoffroy, 1785 according to Vives, 2000)

Unavailable name: *Cerambyx parvus tigriformis* Voet, 1778: 7

Here considered as a trinomen and therefore outside zoological nomenclature.

Comments: No type(s) have been recovered of any of the synonyms, and we regard the type material as lost (cf. Horn & Kahle 1935, Horn et al., 1990). Both Goeze (1777) and Gmelin (1790) introduced binominal names by quoting the same description by Lepechin (1775). *Cerambyx taeniatus* is therefore a junior synonym of *Cerambyx bifasciatus*. The description of *Cerambyx bifasciatus* (e.g. antennae twice as long as the body) does not correspond to the original description of *L. nebulosus*, and the drawing in Lepechin (1775) is not detailed enough to confirm that it belongs to *Leiopus*. *Cerambyx niger* was considered to be a synonym by Olivier (1789–1808) and Gmelin (1790), and *C. monilis* was considered to be a synonym by Breuning (1963) and Villiers (1978). However, *C. monilis* and *C. niger* are almost 3 times larger (3 ½ lig.) than *C. nebulosus* (1 ⅔ lig.) in the descriptions by Geoffroy (1762, 1785). The description of *Cerambyx fasciatus* is based on a drawing (Villers, 1789), which is not detailed enough to confirm that it belongs to *Leiopus*. There is a detailed and accurate drawing in Voet (1778) that corresponds to *L. nebulosus*, and not *L. sp. nov.*

However, we have not designated a neotype of *Cerambyx parvus tigriformis*, since we consider it to be an unavailable name (ICZN). The conclusion is that we do not find any published name to be available for *Leiopus* **sp. nov.**

New synonyms: Examination of colour photos of type material and the key presented by Villiers (1978), and the dissection of a male specimen (the type of *L. nebulosus* var. *siculus* Pic, 1924) indicate that the following are synonyms of *L. nebulosus*:

Leiopus nebulosus var. *dissimilis* Pic, 1889: 5. **syn. nov.**

Leiopus nebulosus var. *unifasciatus* Pic, 1891: 23. **syn. nov.**

Leiopus nebulosus var. *siculus* Pic, 1924:22. **syn. nov.**

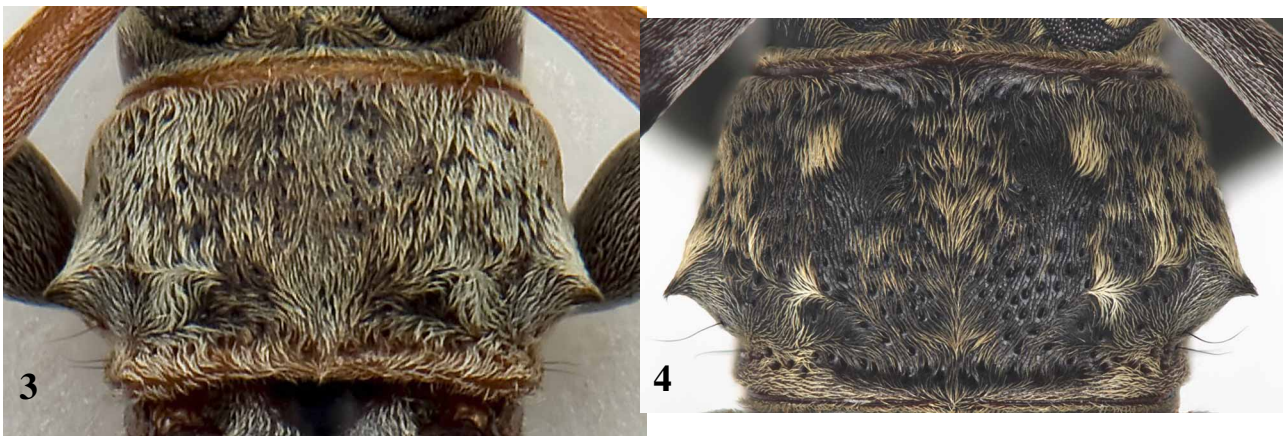
Type material examined: *L. nebulosus* var. *siculus* Pic (Italy, Sicily, 1 male, Lectotype designated by Biscaccianti 2005, MNHNP).



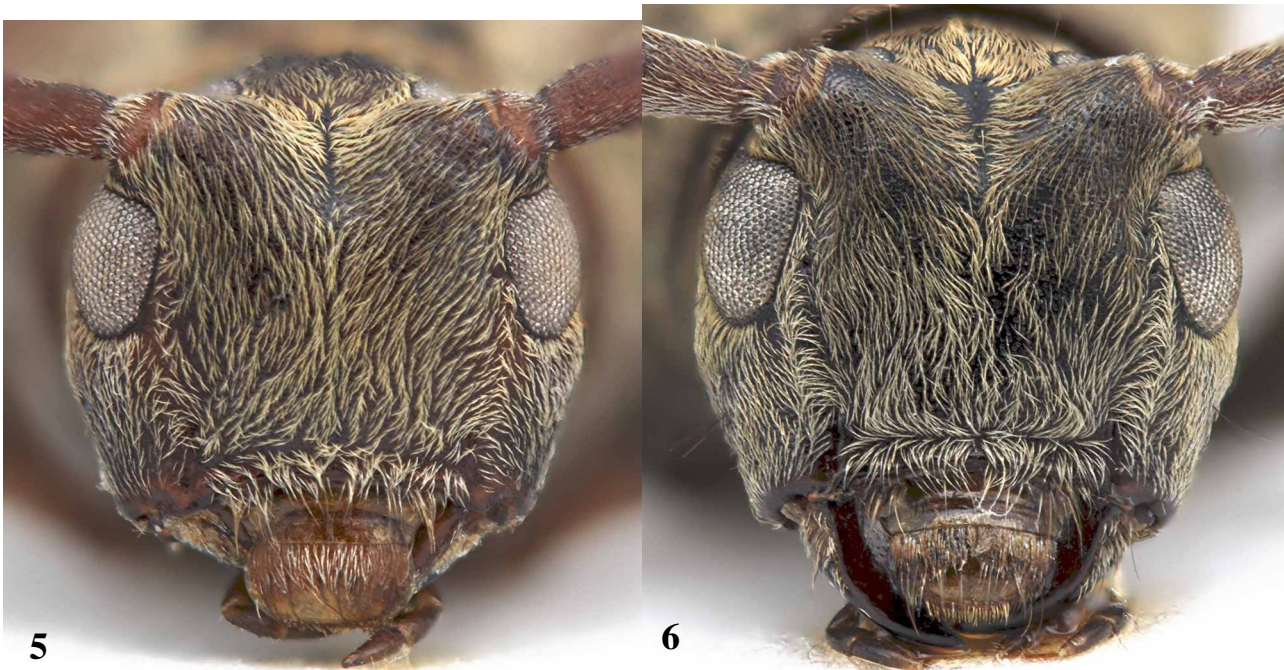
FIGURES 1–2. Habitus. 1, *Leiopus nebulosus* (L.), male, 7.5 mm; 2, *L. linnei* **sp. nov.**, holotype, male, 8.3 mm.

Redescription: Moderate-sized body, subdepressed, similar in shape to *L. linnei* **sp. nov.** (Fig. 1). Body length: 5.2–8.8 mm in males, and 5.9–9.0 mm in females. Elytra covered with whitish pubescence and irregular blackish spots on entire surface, forming an oblique or irregular blackish band near base and, most often, a distinct blackish, transversal band behind middle (Fig. 1). The whitish area on the middle of elytra, between the anterior and posterior dark bands, often covered with a few small dark spots. Pronotum uniformly greyish with no apparent pattern of yellowish spots medially, narrow, flattened, and tubercles oblique or absent (Fig. 3). Pronotal punctuation evenly scattered and relatively fine. Frons on head narrow (Fig. 5). Posterior margin of last visible abdominal segment (ovipositor) of female with very fine and short pubescence (Fig. 7). **Aedeagus** (Fig. 9): approx. 1.2 mm long, relatively slender, narrowed and curved towards apex,

ventral ridge forming a smoothly rounded apex or at most with an obsolete tip. Dorsal ridge with a rounded apex. Microscopic sclerites inside the internal sac forming a circular head and a “V-shaped” structure, and attached to a relatively long straight shaft with a “T-shaped” end, corresponding well to drawings in Sama (1985) and Biscaccianti (2005). **Parameres** (Figs. 11 & 13): approx. 1.7 mm long, rounded dorso-ventrally, slightly separated medially along inner margin and rounded towards apex and often projecting inwards at apex. Apex evenly round along entire posterior margin, with fringes of relatively short, brownish hairs well concentrated at edge of apex, only scattered single hairs medially towards apex. No micro-reticulation on parameres. Base of tegmen, at lower part of lateral lobe, extended and slightly twisted dorso-ventrally on middle. **Proctiger** (the 8th tergum; Fig. 15): approx. 0.8 mm long and covered with short, very fine yellowish or brownish hairs distally towards posterior margin, except for a more or less naked area or only with few scattered hairs medially and narrowing towards the posterior margin. Surface shining medially, with no distinct micro-reticulation. Only weak and scattered micro-reticulation laterally. Posterior margin rounded or only slightly elongated. **Spermatheca** (Fig. 17): yellow to brown, relatively long and slender, not widened at base, and only slightly curved towards apex. Apex with an elongated head.



FIGURES 3–4. Pronotum. 3, *Leiopis nebulosus* (L.); 4, *L. linnei* sp. nov.



FIGURES 5–6. Heads with frons. 5, *Leiopis nebulosus* (L.); 6, *L. linnei* sp. nov.



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FIGURES 7–8. Female abdominal ventrite (ovipositor). 7, *Leiopus nebulosus* (L.); 8, *L. linnei* sp. nov.



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FIGURES 9–10. Aedeagi. 9, *Leiopus nebulosus* (L.); 10, *L. linnei* sp. nov. (arrow shows the extended posterior margin with a distinct pointing edge).



FIGURES 11–14. Parameres. 11, *Leiopus nebulosus* (L.) dorsal view (arrow shows the twisted base of tegmen); 12, *L. linnei* sp. nov. dorsal view; 13, *L. nebulosus* (L.) ventral view; 14, *L. linnei* sp. nov. ventral view.



FIGURES 15–16. Male proctiger (8th tergum). 15, *Leiopus nebulosus* (L.); 16, *L. linnei* sp. nov.

Leiopus linnei sp. nov.

Type locality: Sweden, the province of Uppland, Övergran, Biskops-Arnö.

Holotype: 1 male (body length: 8.3 mm, width: 2.8 mm), Sweden, Province Uppland, Biskops-Arnö, 2007-05, reared from oak branches, H. Wallin leg. The holotype is deposited in the type collection at the Museum of Evolution, Uppsala University (UUZM) with identity = UUZM Types No. 2824a.

Paratypes: (8 males, 6 females): 1 female, Sweden, UP, Biskops-Arnö, 2007-05, reared from oak branches, H. Wallin leg, UUZM Types No. 2824b; 1 male, Sweden, SM, Hornsö, Alsterhus, 1980-05-22, Bengt Andersson leg, reared from oak branches, BA; 1 male, Norway, Ås (AK6), 1977-04-22, reared from oak branches, T. Kvamme leg, NMHO; 1 male, Sweden, SM, Strömserum, 1971-07-09, U. Nylander leg., UN; 1 male, Sweden, UP, Biskops-Arnö, 2007-05, reared from oak branches, H. Wallin leg, HW; 1 male Denmark, Jylland (SJ), Løgumkloster, Løgumbjerg, 1956-06-22, G. Israelson leg, ZML; 1 male, Sweden, SK, Örup, 1979-07-22, A. Dufberg leg., AD; 1 male, Sweden, Holmsjö, BL, 1980-06-24, O. Nodmar leg, ON; 1 male England, Barcombe, Devon, 1924-07-04, oak (ex coll. C.J. Saunders), BMNH; 1 female, Sweden, SM, Hornsö, 1974-05-14, Bengt Andersson leg, reared from oak branches, BA; 1 female, Norway, Arendal (AAY), Fløystad kirke, 2006-06-21, S. Olberg leg, NMHO; 1 female, Sweden, SM, Strömserum, 1971-07-09, U. Nylander leg, UN; 1 female Denmark, Jylland (SJ), Løgumkloster, Løgumbjerg, 1956-06-22, G. Israelsson leg, ZML; 1 female England, Barcombe, Devon, 1924-07-04, oak (ex coll. C.J. Saunders), BMNH.

Description: Moderate-sized, subdepressed, similar in shape to *L. nebulosus* (L.) (Fig.2). Body length: 5.0–9.2 mm in males, and 6.4–9.5 mm in females. Entire surface covered with whitish pubescence with irregular blackish spots, forming an irregular and broad blackish band near base, and most often, an oblique to blackish band behind middle (Fig. 2). A whitish area on the middle of the elytra, between the anterior and posterior dark bands, often with numerous small dark spots. Pronotum covered with a mixture of brownish and yellowish pubescence forming a pattern of lighter spots or transversal bands medially (Fig. 4). Pronotum is wide, often with three to five raised tubercles: two close to the posterior margin, one longitudinally elongated medially, and two close to the anterior margin. Area between four marginal tubercles often covered with yellowish pubescence. Pronotal punctuation relatively coarse and more concentrated medially. Frons more protruding and wider than that of *L. nebulosus* (Fig. 6). Maximum width of frons (mean \pm SD: 1.23 mm \pm 0.16; n=20) significantly greater than that of *L. nebulosus* (0.98 mm \pm 0.13, n=20) (t-test, $P < 0.0001$). In order to eliminate any influence of body size, an index (maximum width of frons/maximum width the right eye) was used, which also resulted in significant differences (t-test, $P < 0.0001$). Posterior margin of last visible abdominal segment (ovipositor) of female covered with a dense plume of long hairs (Fig. 8). Body length of examined males (7.83 mm \pm 0.88, n=87) significantly greater than that of *L. nebulosus* males (7.05 mm \pm 0.74, n=87) (t-test, $P < 0.0001$). Examined females significantly longer (8.21 mm \pm 0.70, n=61) than *L. nebulosus* females (7.39 mm \pm 0.75, n=65) (t-test, $P < 0.0001$). **Aedeagus** (Fig. 10): approx. 1.7 mm long, relatively broad and stout, slightly widened to the middle and thereafter only slightly narrowed and curved towards apex, ventral ridge protruding, forming a distinct apical tip, often elongated and pointing ventrally. Dorsal ridge with a rounded apex. Microscopic sclerites inside internal sac forming a circular head without a "V-shaped" structure and a "T-shaped" end found in *L. nebulosus*. **Parameres** (Figs. 12 & 14): approx. 1.8 mm long, flattened dorso-ventrally, not separated, forming a straight inner line towards apex, apex with sharp edge inwards and more rounded laterally; with a mixture of fine and coarse fringes of relatively short, brownish hairs well concentrated at edge of apex, also scattered single hairs medially towards apex. Basal part of parameres protruding, giving it a swollen appearance. Surface medially along inner margin and predominantly towards apex often covered with weak transversal micro-reticulation. Base of tegmen at lower part of lateral lobe extended and forming a straight line dorso-ventrally, and not twisted in the middle. **Proctiger** (8th tergum; Fig. 16): approx. 1.0 mm long and densely covered with long coarse yellowish, brownish or black hairs distally towards posterior margin, covering entire surface medially. Surface not shining medially, with distinct square-formed micro-reticulation both medially and laterally. Posterior margin extended and often forming a distinct pointing edge, almost triangular. **Spermatheca** (Fig. 17): yellow to

brown, relatively short and stout, widened at base, and acutely curved towards apex. Apex with an elongated head.

Comments: The genitalia characteristics of both sexes amongst all dissected specimens of the two sibling species of *Leiopus* were found to be stable. We found no similarities in the genitalia characters between *L. linnei*, and any of the examined Palaearctic species of *Leiopus*. The male genitalia characters of *L. insulanus*, are similar to those described for *L. nebulosus* but not *L. linnei*.

Etymology: This species is named in honour of Carl von Linné, who introduced the binary nomenclature. The final part of the work was made during 2007, which corresponds with the 300th anniversary of Linnaeus's birth. *Cerambyx nebulosus* was described by Linnaeus and this is an additional reason to dedicate the name of the sibling species to him.



FIGURE 17. Spermathecae (scanning electron micrographs). *Leiopus linnei* **sp. nov.** (left) and *L. nebulosus* (right).

Key to the two sibling species of *Leiopus*

- Frons narrow and not protruding (Fig. 5). Posterior margin of last visible ventrite of females with very fine and short pubescence (Fig. 7). Aedeagus with ventral ridge forming a smoothly rounded apex or at the most with an obsolete tip (Fig. 9), parameres rounded dorso-ventrally and towards apex, base of tegmen extended and slightly twisted dorso-ventrally on middle (Figs. 11 & 13). Proctiger covered with short, very fine hairs distally towards posterior margin, except for a more or less naked area medially (Fig. 15). Spermatheca relatively long and slender and not widened at base (Fig. 17). *L. nebulosus*
- Frons wide and protruding (Fig. 6). Posterior margin of last visible ventrite of females with a dense plume of long hairs (Fig. 8). Aedeagus with ventral ridge protruding and forming a distinct apical tip, often elongated and pointing ventrally (Fig. 10), parameres flattened dorso-ventrally forming a straight inner line towards apex, apex with a sharp edge inwards and more rounded laterally, base of tegmen extended and forming a straight line dorso-ventrally, and not twisted in the middle (Figs. 12 & 14). Proctiger covered with dense, long, coarse hairs distally towards posterior margin, covering entire surface medially (Fig. 16). Spermatheca relatively short and stout and widened at base (Fig. 17). *L. linnei* **sp. nov.**

DNA-analysis: The result of the DNA barcoding analysis shows that the percentages in genetic distance between the three examined species of *Leiopus* are significant (Table 1). The neighbouring joining tree (Fig. 18) depicts the relationship between the species. A sistergroup relationship was found between *L. nebulosus* and *L. linnei* (Fig. 18). *L. nebulosus* and *L. linnei* constitute a pair of morphological siblings. The great genetical difference between the species, combined with the morphological difference, justifies the description of *L. linnei* as a genuine species. We interpret the substantial genetical difference as a long standing isolation of the two sibling species. The similarity in choice of habitat and geographical distribution is possibly a result of the two sibling species following parallel paths during the process of evolution.

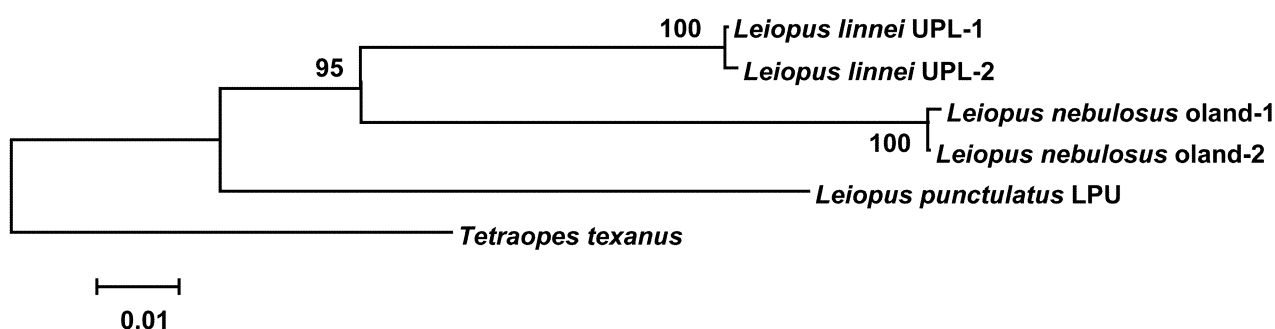


FIGURE 18. DNA-analysis: neighboring joining tree. The relationship between *Leiopus nebulosus* (L.), *L. linnei* sp. nov. and *L. punctulatus* (Paykull). *Tetraopes texanus* Horn was used as an outgroup.

TABLE 1. DNA-analysis: distance-matrix showing the differences as percentage. The GenBank numbers for the sequences are added. The abbreviations used are LPU= *Leiopus punctulatus* (Paykull), UPL-1 and UPL-2= *L. linnei* n. sp., and oland-1 and oland-2= *L. nebulosus* (L.). *Tetraopes texanus* Horn was used as an outgroup.

| | <i>Tetraopes texanus</i> | <i>Leiopus</i> LPU | <i>Leiopus</i> UPL-1 | <i>Leiopus</i> UPL-2 | <i>Leiopus</i> oland-1 | <i>Leiopus</i> oland-2 |
|--------------------------|--------------------------|--------------------|----------------------|----------------------|------------------------|------------------------|
| <i>Tetraopes texanus</i> | | | | | | |
| <i>Leiopus</i> LPU | 0.15052 | | | | | |
| <i>Leiopus</i> UPL-1 | 0.13841 | 0.13495 | | | | |
| <i>Leiopus</i> UPL-2 | 0.14014 | 0.13668 | 0.00173 | | | |
| <i>Leiopus</i> oland-1 | 0.16782 | 0.15744 | 0.11592 | 0.11592 | | |
| <i>Leiopus</i> oland-2 | 0.16782 | 0.15571 | 0.11419 | 0.11419 | 0.00173 | |

The GenBank accession numbers provided for the nucleotide sequences:

Leiopus LPU (= *Leiopus punctulatus*) GenBank no. EU436850

Leiopus UPL-1 (= *Leiopus linnei* sp. nov.) GenBank no. EU436851

Leiopus UPL-2 (= *Leiopus linnei* sp. nov.) GenBank no. EU436852

Leiopus Oland-1 (= *Leiopus nebulosus*) GenBank no. EU436853

Leiopus Oland-2 (= *Leiopus nebulosus*) GenBank no. EU436854

Biology: Larvae of *L. nebulosus* were reared from branches of *Corylus avellana*, between 2 and 6 cm in diameter, with rather thin bark. The larvae make galleries tight under the bark in the cambial layer, with finely structured frass. The tunneling slightly penetrates the sapwood (Fig. 19A). Pupal chambers are made by widening the gallery and are usually positioned between bark and sapwood, often close to twigs (Fig. 19B).

Larvae of *L. nebulosus* develop in branches with relatively low moisture. Examined specimens have also been reared from *Quercus* and *Tilia* in Sweden, from *Juglans* in France and from *Ficus*, *Alnus*, *Fagus*, *Prunus* and *Corylus* in Italy. The preferred host tree in Scandinavia appears to be *Corylus*.

The larvae of *L. linnei* make galleries by mining under the thick bark of *Quercus* or *Acer* as observed in Sweden. The frass made by these larvae is much coarser compared to the frass of *L. nebulosus*. The development takes place in branches with relatively high moisture. The pupal chamber is a shallow excavation between the bark and wood (Fig. 19C), or even in the bark when this is very thick. The larvae construct a ring of coarse frass around the pupal chamber and close the exit hole with packed frass (Fig. 19C). Pupal chambers can also be excavated in the sapwood when branches are less than 5 cm in diameter (Fig. 19D & E). Examined specimens of *L. linnei* have been reared from a wide range of host trees (cf. Ehnström & Axelsson 2002; Sama 2002).

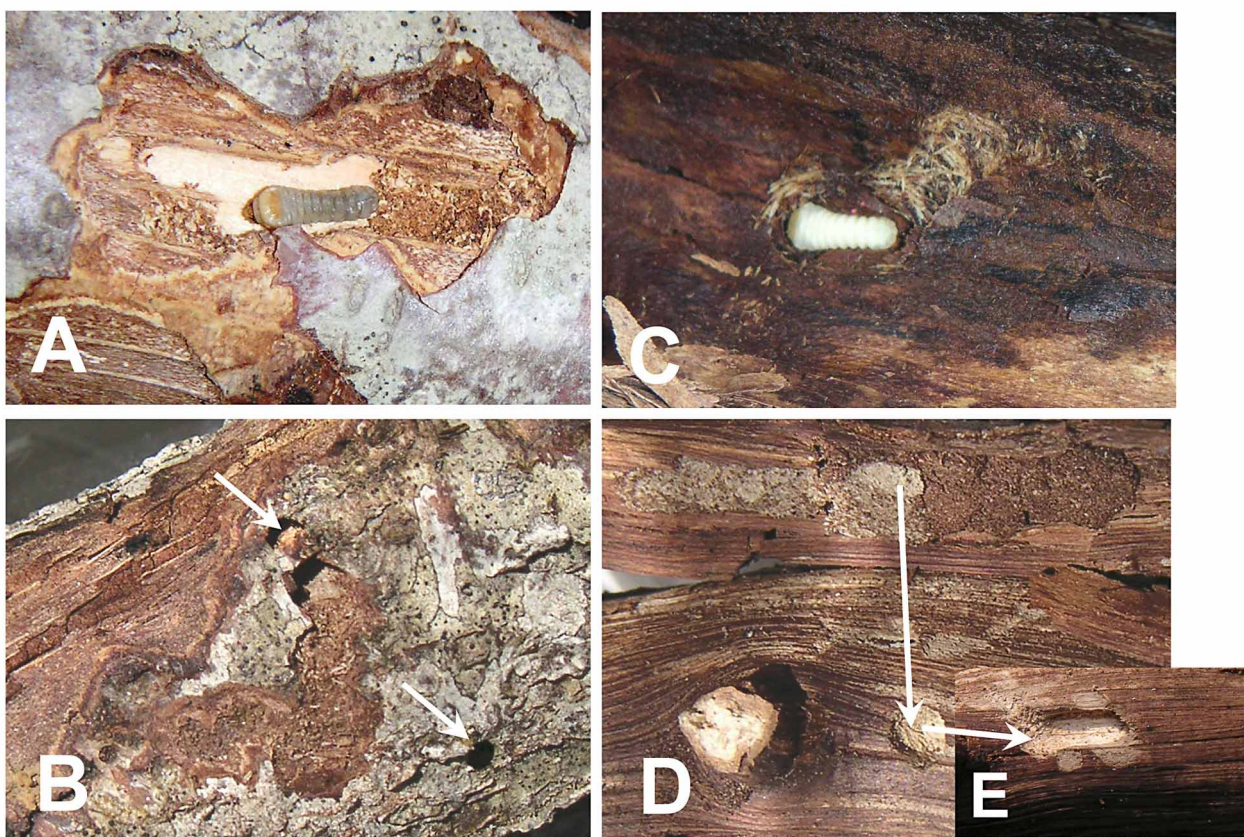


FIGURE 19. Larval galleries and pupal chambers. A, larval galleries of *Leiodus nebulosus* (L.) under thin bark of *Corylus avellana* from Sweden; B, exit holes and a partly exposed pupal chamber of *L. nebulosus* (arrows); C, larval gallery and pupal chamber of *L. linnei* **sp. nov.** under thick bark of *Quercus robur* from the type locality area in Sweden; D, larval gallery and pupal chamber of *L. linnei* **sp. nov.** under bark of thinner branches of *Quercus robur* from the type locality area; E, exposed pupal chamber excavated into the sapwood.

Geographical distribution: *L. nebulosus* is predominantly recorded from coastal areas and islands in Scandinavia (Fig. 20). It is the only sibling species recorded from any of the islands in the Baltic Sea, or from Finland (coastal areas in SW Finland; cf. Heliövaraara, Mannerkoski & Siitonen 2004). There is only one inland record in Sweden (Fig. 20). The material we have examined include specimens of *L. nebulosus* from central France (Grenoble) and Germany (near Berlin), and numerous records from southern Italy, including Sicily. The species is also recorded from Ireland and the British Isles: from southern England to Scotland.

L. linnei is common and widely distributed in Norway, Sweden and Denmark in Scandinavia (Fig. 20). The collection data show numerous inland records from especially Sweden (Fig. 20). Remaining records are from

the British Isles, France, Poland, Czech Republic, Slovakia, Austria, Croatia, Bulgaria and Rumania. The species appears to be common in central Europe (inland habitats). Sympatric populations of the two sibling species mainly occur in coastal areas in Norway, Denmark and Sweden (Fig. 20). There are also indications of several mixed, or sympatric, populations of *L. nebulosus* and *L. linnei* in southern England, from the material we have examined.

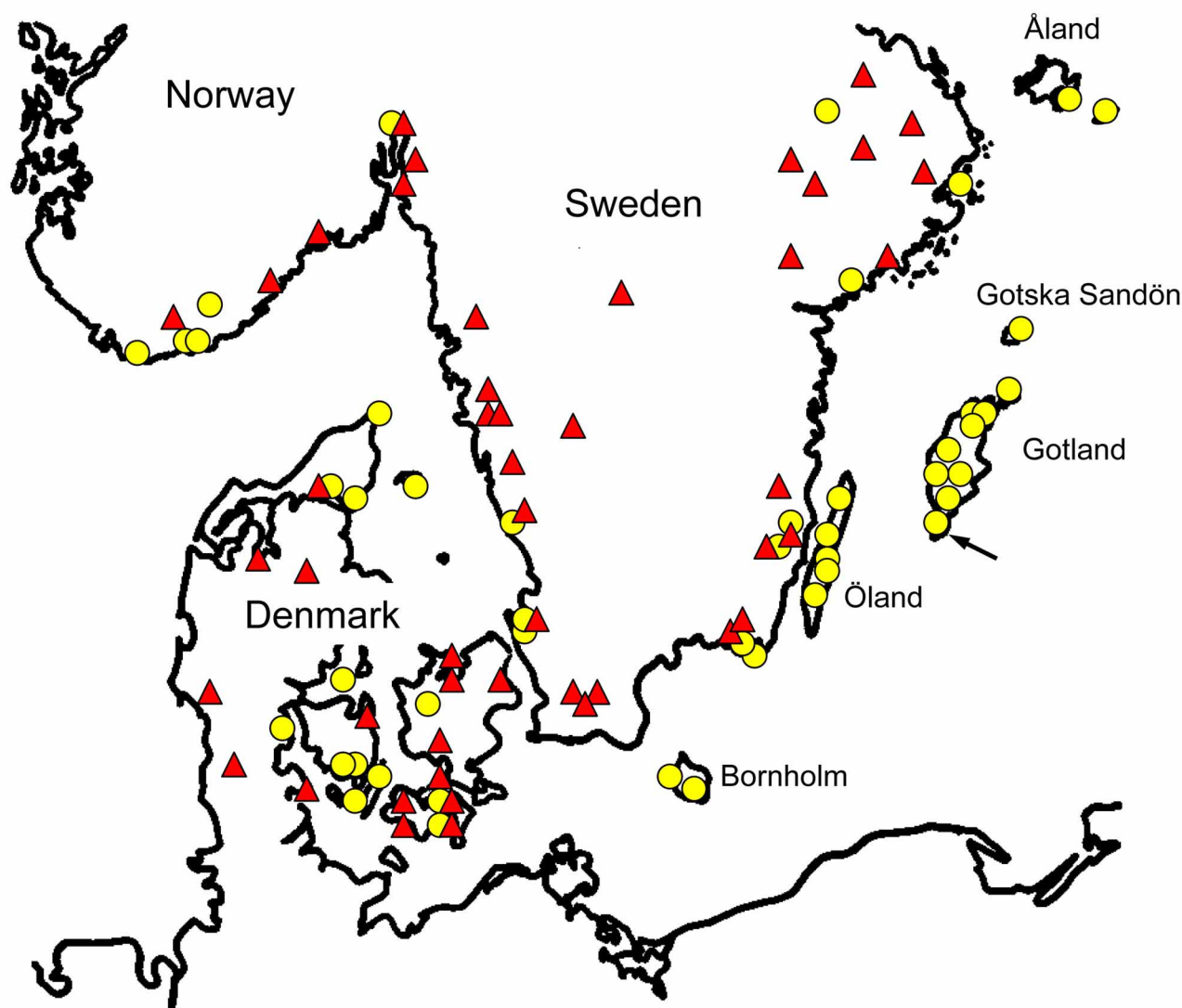


FIGURE 20. Distribution of records from Scandinavia. Yellow circles, *Leiopus nebulosus* (L.); red triangles, *L. linnei* sp. The arrow shows the type locality for *L. nebulosus* (Sweden, the island of Gotland, Burgsvik). Overlapping symbols (circles/triangles) show records that indicate the presence of sympatric populations.

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