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Hind wing morphology facilitates discrimination between two sibling species: *Leiopus nebulosus* and *L. linnei* (Coleoptera: Cerambycidae)

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Abstract

The study focused on two sibling beetle species: *Leiopus nebulosus* (Linnaeus, 1758) and *L. linnei* Wallin, Nylander & Kvamme, 2009. These species are very similar morphologically and their identification is difficult and possible only by experienced taxonomists. A supporting method for identification of *L. nebulosus* and *L. linnei* based on hind wings measurements was developed. The study was based on 115 specimens of *L. linnei* and 45 specimens of *L. nebulosus*. The correctness of identification of *L. nebulosus* amounted to 95.56%, and of *L. linnei* – 97.39%. The obtained model facilitates reliable identification of *L. nebulosus* and *L. linnei* also by less experienced entomologists. Geographical distributions of both species were summarized based on faunistic data from 39 scientific papers. The results show that both species have a Western-Palearctic distribution. Their distribution ranges are markedly overlapping. However, *L. linnei* is the species reported from larger number of localities, and observed more often.

Key words: *Leiopus linnei*, *Leiopus nebulosus*, sympatric species, geographical distribution, species identification, morphological characters, taxonomy

Introduction

The genus *Leiopus* Audinet-Serville, 1835 occurs throughout most of the Palaearctic region (Löbl & Smetana 2010; Danilevsky 2012). After the last review (Wallin *et al.* 2012), the genus comprises 26 species grouped in two subgenera. Subgenus *Carinopus* Wallin, Kvamme & Lin, 2012 represents six species distributed in China. The remaining 17 species of subgenus *Leiopus* Audinet-Serville, 1835 occur throughout most of the Palaearctic region. Five species occur in Europe: *L. femoratus* Fairmaire, 1859, *L. linnei* Wallin, Nylander & Kvamme, 2009, *L. nebulosus* (Linnaeus, 1758), *L. punctulatus* (Paykull, 1800) and *L. settei* Sama, 1985 (Danilevsky 2012). *Leiopus linnei* and *L. nebulosus* were separated after a detailed morphological and genetic analyses (Wallin *et al.* 2009). However, they demonstrate great morphological similarity. The two species are called "*nebulosus* species group" in the presented paper. Separation of *L. nebulosus* and *L. linnei* is possible only by comparing the male genitalia and the sclerotized part of the spermatheca in females. Comparing the shape of head and of the pygidium could also serve as auxiliary features (Wallin *et al.* 2009). Nevertheless, it should be noted that the abovementioned features show great individual variability. It is thus necessary to revise the whole material of the *L. nebulosus*-group identified prior to the description of *L. linnei* in order to understand the distribution of the two species.

Most of the studies on *Leiopus* spp. distribution published in last seven years focus mainly on areas valuable in terms of nature (Kozak 2010a, 2010b; Nakládal 2011a, 2011b; Kašák *et al.* 2012; Zamoroka *et al.* 2012; Ilić & Ćurčić 2013; Blick & Dorow 2014; Olbrycht 2014; Egorov *et al.* 2015). Detailed national studies were completed only for some countries e.g. Ireland (Alexander & Anderson 2012), Latvia (Telnov *et al.* 2010; Barševskis & Savenkov 2013), Macedonia (Plewa *et al.* 2015), Norway (Kvamme & Wallin 2013), Poland (Gutowski *et al.* 2010), Russian Federation–Kaliningrad region (Alekseev & Bukejs 2011), Serbia (Ilić & Ćurčić 2015), Sweden

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(Wallin *et al.* 2009) and Ukraine (Zamoroka *et al.* 2012). Some studies only indicate that the second species might be present (Cuppen & Drost 2009; Cuppen 2012) and only show the general range of distribution of the *nebulosus* species-group.

The objective of our study was to facilitate the identification of *L. nebulosus* and *L. linnei*, based on the measurements of hind wings. Moreover, on the basis of available material and 39 studies published after 2009, the geographical distribution of both species is summarized.

Material and methods

Examined material. The study was based on 160 imagines of *Leiopus linnei* (64♀, 51♂) and *L. nebulosus* (25♀, 20♂). The specimens were identified based on the identification key of Bense (1995) and Wallin *et al*. (2009). The full label data for each specimen are presented as name of country and locality, UTM code (Universal Transverse Mercator wgs84, 10x10 square km grids), when possible, collection date, collector and identifier, deposition, number of specimens and sex. When possible, the names of host plants are given from which the specimens where collected. The following abbreviations were used:

AT—Andrzej Trzeciak; HS—Henryk Szołtys; JF—J. Frunze; JP—Jiři Plecháč; PD—P. Dziedzic; RR—Robert Rossa; SO—Sebastian Ostaszewski; TB—Tadeusz Bziuk; TS—Tomasz Śmiałek; UR—Collection at the University of Agriculture in Krakow.

Leiopus nebulosus

Czech Republic—Bohemia: Bělohrad, Byšička, Lažne, WR48, 10.VI.1988, leg. JP, det. RR, coll. UR, 1♂; Nižbor, VR23, 31.VII.1984, leg. JP, det. RR, coll. UR, 1♂; Pecka, Podkrkonoši, WR49, 29.VI.1999, leg. JP, det. RR, coll. UR, 1♂; Příbram, VR20, 22.VII.1987, leg. JP, det. RR, coll. UR, 1♀; **Poland—**Bolęcin ad Chrzanów, CA95, 20.III.2014, leg. TS, leg. RR, coll. UR, 5♂♂, 4♀♀, ex cult. *Alnus* sp.; Brynek, CA39, 8.V.2014, leg. et det. HS, coll. UR, 1♀; Dębica, EA34, 6.V.2005, 11–20.IV.2013, leg. AT, det. RR, coll. UR, 2♀♀, ex cult. *Quercus* sp.; Jurczyce, UTM(?), 13.VI.2011, leg. et det. JF, coll. UR1♀; Kalety, CB50, 15.VI.2013, leg. et det. P. Kocot, coll. UR, 1♂; Miedary, CA39, 5.VII.2014, 4.VI.2015, leg. et det. HS, coll. UR, 2♀♀; Pogórska Wola, EA14, VII.2015, leg. ?, det. RR, coll. UR, 2♀♀; Pusta Kuźnica, CA30, 24.IV.2010, leg. TB, det. RR, coll. UR, 1♂, 3♀♀, ex cult.; Puszcza Białowieska, FD74, 20.VI.2005, leg. SO, det. RR, coll. UR, 1♀, branches of *Quercus* sp.; Ruda Śląska, CA46, 6.VI.2010, 1♂, 10.VI.2011, 3♂♂, 1♀, 1.VI.2013, 1♂, 1♀, leg. TB, det. RR, coll. UR, ex cult.; Ruda Śląska, CA56, 26.V.2015, leg. TB, det. RR, coll. UR, 1♀; Słupsko, CA28, 12.VI.2011, leg. TB, det. RR, coll. UR, 1♂, 1♀; Sprzęcice, BA99, 15.III.2015, leg. TB, det. RR, coll. UR, 1♀, ex cult.; Wygiełzów ad Chrzanów, CA84, 5.V.2014, leg. TS, det. et coll. RR, 2♂♂, 3♀♀, ex cult. *Juglans regia*; **Slovakia—**Nízké Tatry, Dolina Hronec, Závodka nad Hronom, DV21, 30.VII–1.VIII.2010, leg. JP, det. RR, coll. UR, 1♂.

Leiopus linnei

Czech Republic—Bohemia: Bělohrad, Byšička, Lažne, WR48, 10.VI.1988, leg. JP, det. RR, coll. UR, 1♀; Pecka, Podkrkonoši, WR49, 19.VI.1995, 18.VII.2013, leg. JP, det. RR; coll. UR, 2♂♂; Sedlčany env., VR50, 28.VI.2011, leg. A. Trmal, det. RR, coll. UR, 1♀; Moravia: Jevišovická pahorkatina, Hartvikovice p. Mohelno, WQ74, 3.VI.2011, leg. JP, det. RR, coll. UR, 1♀; **Poland—**Brynek, CA39, 4.VII.2009, 1♀, 1.VIII.2012, 1♂, 8.VIII.2013, 3♀♀, 24.V.2014, 4♂♂, 5♀♀, 29.VI.2014, 1♀, leg. HS, det. RR, coll. UR; Czchów res., DA72, 24.VIII.2013, leg. PD, det. RR, coll. UR, 1♀; Dębica, EA34, 6.V.2005, 1♂, leg. AT, det. RR, coll. UR; Stasiówka, EA34, 20–27.V.2011, leg. AT, det. RR, coll. UR, 1♀, ex cult.; Grzybów, UTM (?), 12.V.2013, leg. et det. JF, coll. UR, 1♀; Jawor, WS85, 22. et 25.V.2013, 1♂, 1♀, 12.VI.2013, 1♂, 1♀, leg. ?, det. RR, coll. UR; Kalety, CB50, 23.VI.2011, 1♀, 15.VI.2013, 2♂♂, leg. et det. P. Kocot, coll. UR; Karpniki, WS53, 7.VI.2015, leg. ?, det. RR, coll. UR, 1♂; Legnica, WS77 et WS87, 12. et 15.VI.2011, leg. ?, det. RR, coll. UR, 5♂♂, 1♀; Mętków, CA84, 30.VI.2013, 1♀, Płaza, CA84, 4.VII.2013, 1♂, leg. P. Mąsior, det. RR, coll. UR; Miedary, CA39, 5.VII.2014, 1♀, 4.VI.2015, 1♂, leg. et det. HS, coll. UR; Puszcza Białowieska, FD74, 20.VI.2005, leg. SO, det. RR, coll. UR, 1♀, branches of *Quercus* sp.; Puszcza Niepołomicka, DA54, 29.V.2012, leg. E. Siedlarczyk, det. RR, coll. UR, 1♀, 1.VIII.2013, leg. PD, det. RR, coll. UR, 1♀; 7.VI.2014, leg., det. et. coll. RR, 1♂, 1♀; Ruda Śląska, CA46, 6.VI.2010, 1♂, 10.VI.2011, 8♂♂, 4♀♀, 1.VI.2013, 2♀♀, leg. TB, det. RR, coll. UR, ex cult.; Ruda Śląska, CA56, 10.II.2015, 1♀, leg. K. Doroz, det. RR, coll. UR; 5.I.2015, 1♀, 15.IV.2015, 1♀, 10.V.2015, 1♂, 3♀♀, leg. TB, det.

RR, coll. UR, ex cult.; Słupsko, CA28, 15.IV.2012, leg. TB, det. RR, coll. UR, 13♂♂, 16♀♀, ex cult.; Sprzęcice, BA99, 15.III.2015, leg. TB, det. RR, coll. UR, 3♂♂, 3♀♀, ex cult.; Ścinawa, WS99 et XS09, 13.V.2013, 1♂, 2♀♀, 11.VI.2013, 2♂♂, 3♀♀, leg. ?, det. RR, coll. UR; Wołów, XS18, 7.VI.2013, leg. ?, det. RR, coll. UR, 1♂, 3♀♀; **Slovakia—**Jurský Chlm, CT19, 15.VI.1995, leg. JP, det. RR, coll. UR, 1♀.

Species distribution ranges. To summarize the known distribution of *L.* nebulosus and *L. linnei,* the faunistic data from publications following the description of *L. linnei* in 2009 were used:

Leiopus linnei

Austria: Baden ad Wien, WP91, Perchtoldsdorf ad Wien, WP93 (Gutowski *et al.* 2010), Bad Gleichenberg, WM69, Graz, WN31, Anger, WN53, Herberstein, WN62, Stubenberg, WN63, Neudau, WN82 (Holzer 2014); **Belarus:** Białowieża Primeval Forest, UTM(?), Grodno, FE85 (Gutowski *et al.* 2010); **Bulgaria:** Rabisha, FP24 (Gutowski *et al*. 2010); **Czech Republic:** Mladeè, XR40 (Nakládal 2011a), Zástudánčí, XQ67 (Nakládal 2011b), Borek u Domašova, XR55 (Kašák *et al*. 2012); **Denmark:** Løgumkloster, Løgumbjerg, MG90 (Wallin *et al*. 2009); **Estonia:** Keblaste, LF30, Kuusnõmme, EK56, Luidja, EL73-83 (Bukejs & Balalaikins 2011); **Germany:** Forest Reserve "Kinzigaue", NA05, Hessen, UTM (?) (Blick & Dorow 2014); **Latvia:** Aizkraukle, LC97, Bērzciems, FJ24, Cekule vill., LD41, Dunika PNT, EH23, Gauja NP, Krimulda, Sigulda, LD63-73, Īslīce PNT, LC25, Kandava, EJ92, Ķemeri NP, FJ41, Moricsala Isl., EJ63-64, Ozoldārzs, LC15, Paņemūnes PNT, LC(?), Reņģe, FH05, Rīga city, LD21, Rundāle, LC15, Sabile, EJ91, Silene PNT, Ilgas, MB87, Slītere NP, EJ88, Vilce, FH55, Vestiena PNT, near Lake Vidusezers, MD30, (Telnov *et al*. 2010), **Lithuania:** Wilno, LA86 (Gutowski *et al*. 2010); **Luxembourg:** Haardt NR, KV88 (Vitali 2014); **Macedonia:** Leskoec, DL85 (Plewa *at al*. 2015); **Norway:** Moss: Jeløya, NL99, Råde: Tasken, PL07, Fredrikstad: Rauøy, NL96, Bærum: Stabekk, NM84, Nesodden, NM93, Oslo: Hurum, NM80, Oslo, NM94, Tønsberg: Jarlsberg, NL77, Kragerø: Jomfruland, NL32, Knipheia, NL22, Birkenes, MK57, Kristiansand: Bråvann, MK34, Nedre Timenes, MK44, Sør-Audnedal, MK06, Vigeland, LK93-MK03, Lyngdal: Nebbdal, LK84, Mandal, MK03, Vennesla, MK35-45, Nord-Fron: Stordalsberget, NP42, Sogndal: Fatlaberget, LN88, Luster: Ornes, MN19, Leikanger: Grindestrondi, LN78, Sula, LQ52, Nesset: Øvre Vike, MQ53, Sunndal: Hoelsand, MQ84, Oppdølstranda, MQ74, Gravem, NQ03, Flåstranda, MQ75, Ottem, MQ74, Melhus, NR61, Trondheim, NR63, Byneset, Almli, NR52-62 (Kvamme & Wallin 2013, 2016); **Poland:** Smoszew, XT72 (Hilszczański & Plewa 2009), Białobrzegi Radomskie, DC92, Białowieża NP, Wysokie Bagno res., FD94, Bielinek res., VU46, Bielnik, DB93, Błeszno, DC91, Błogie, DB29, Chojnów, EC06, Chrzemce, DC31, Cieszyn, CA21, Czerkies res., Obrocz, FB40, Czerlonka, Stara Białowieża, FD84, Czerwony Dwór, EF70, Czeszewski Las res., XT77, Dąbrowa Jastrzębska, Dąbrowa Kozłowska, Jastrzębia, Lesiów, Radom-Wincentów, Wola Owadowska, EC10, Dulowa, CA95, Gacki, DA79, Garbatka Letnisko, EC40, Gołuchów, YT04, Gruszki, FD85, Gutkowo, DE66, Hajnówka, FD74, Henryków, EC12, Henryków, XS41, Ispina, DA55, Jarosław, FA24, Jedlnia res., EB29, Jeleń, DC30, Józefów, DB25, Kampinoski NP, Sieraków res., Warszawa-Bielany, DC99, Klembów res., ED20, Kłaj, DA53, Knyszyńska Forest, FE50, Kołków, DA69, Konewka, DC41, Kosy Most, FD95, Kraków, DA14, Łask, CC71, Łaznów, DC12, Łódź, CC93, Macoszyn, FB79, Majdan Stuleński, FB89, Marianów, EC21, Meszcze, DB19, Michnówka res., FD73, Mierzecin, VV86, Międzyborze, XT54, Międzyzdroje, Wolin NP, VV67, Miszek, CD55, Natolin, EB19, Oborniki Śląskie, XS38, Ojców NP, DA16, Orońsko, DB98, Pawłosiów, FA13, Pionki, Brzeźniczka res., EC30, Pionki res., Poświętne, Załamanek res., EC20, Pobierowo, VV99, Poznań, XU20-21, Poznań-Naramowice, XU31, Promno, XU51, Przemyśl, FA21, Radom, EB08-09, Rogalin, XT38, Romincka Forest, Ostrówko, Pluszkiejmy, EF91, Rudzki Most, XV93, Selwanówka, EC22, Serniawy, FB69, Sękocin, Sękocin Stary, Warszawa-Paluch, DC97, Słonecznik, DE26, Sobów, EB50, Spała, DC40, Spokojna, WT75, Sromowce Kąty, Sromowce Niżne, DV47, Szczaki, DC96, Śmiechowice, XS94, Świetlista Dąbrowa res., XU04, Trzebaw, Wielkopolski NP, Wiry, XT29, Urbanów, EC01, Wałki, EA14, Warszawa-Ursynów, EC07, Warzyce, EA31, Witków, GB10, Wrocław-Wojnów, XS46, Żednia distr., FD68 (Gutowski *et al.* 2010), Chełmowa Góra, EB03 (Kozak 2010a), Hulskie, FV15 (Kozak 2010b), Jaworzno, CB35 (Karpiński *et al.* 2011), Mików, EV86 (Kurzawa *et al.* 2012), Patria Nad Odrzechową—Natura 2000, EV78-79 (Olbrycht 2014), Hajnówka, FD74, Krotoszyn, XT72, Łochów, ED70, Pińczów, DA79, Puławy, EB69 (Plewa *et al*. 2014b), Kampinos National Park, UTM(?), Mazovian Nature Park, UTM(?) (Górski & Tatur-Dytkowski 2015), Głuchów, DC33, Rogów, DC24 (Borowski & Sławski 2016); **Romania:** Bãneasa, MK22, Dumbrava-Sibiu, KL76, Transilvania KL76 (Plewa *et al.* 2014a); **Russian Federation:** Kurchatov, XC82 (Kovalenko 2010); Aleksandrovka, EF38, Chernyakhovsk, EF55-56, Divnoe Lake, DF67, Kolosovka, DF57, Svetlogorsk, DF48 (Alekseev & Bukejs 2011), Leski, DU95-96, EU05-

06 (Mazurov 2011; Mazurov & Mazurov 2014), Les na Vorskle, YB01, Puljaevka, CR39 (Kovalenko & Nikitsky 2013), The Chuvash Republic, UTM(?) (Egorov & Ivanov 2014), Inorskoye, LF86 (Egorov *et al*. 2015); **Sweden:** Biskops-Arnö, XG41, Holmsjö, WC35, Hornsö, WD71, Örup, UB96, Strömserum, WD78 (Wallin *et al.* 2009); **Slovakia:** Slovensky Kras Mts., DU88 (Gutowski *et al.* 2010); **Ukraine:** Brzuchowice, GA13, Crimea-Krasnogvardeiskoe, XR03, Kitayhorod, MP88, Krutiliv, UTM(?), Lesieczniki, MP19, Lomachyntsi, NP28, Lwów, KR82, Obizhova ad Zalishtshyky, MP08, Prelipcze, MP08, Range, Kizi—Czarnohora, LP13-23, Sokolivka, TV93, Tarnopol, LQ98-99, Wroców, GA02 (Gutowski *et al*. 2010), Hutysko, LQ47 (Zamoroka *et al.* 2012); **United Kingdom:** Barcombe, Devon, UTM(?) (Wallin *et al.* 2009).

Leiopus nebulosus

Austria: Wien-Kahlenberg, WP94 (Gutowski *et al*. 2010), Anger, WN53, Bad Gleichenberg, WM69, Klöch, WM77, Lannach, WM29, Neuwagna, UTM(?), Tieschen, WM78, Unterrohr, WN73 (Holzer 2014); **Czech Republic:** Petrohrad, UR85, PR Střela, UR74, Žihle, UR84, (Týr 2011); **Estonia:** Emmaste, EL90, Hullo, FL24, near Hullo, FL34, Luidja, EL73-83, Saaremaa Isl., FK38 (Bukejs & Balalaikins 2011); **France:** Grenoble, UTM(?) (Wallin *et al.* 2009); **Germany:** near Berlin, UTM(?) (Wallin *et al.* 2009), Forest Reserve „Kinzigaue", NA05, Hessen, UTM(?) (Blick & Dorow 2014); **Ireland:** Abbeyleix, PU16, Clara Bog, Charleville Wood, NV90, Clonbrock, NV41, Cloonmore, MU63, Cragmoher Lough, MU96, Cratloe Wood, NU13, Crom Castle Park, PA00, Down near Belfast, UF14, Phoenix Park [Dublin], PV71, Glengarriff, MT63, Glenmore, PU99, Hare Island, NV72, Killarney, MT66, Lickeen Wood, MT36, Lismore, NT77, Lucan Demesne, PV61, Powerscourt, Knocksink Wood, PU89, Rathfarnham, PV70, Roscommon, Mote Park, NV53, Tandragee Castle, PA62, Tynan Abbey Estate, Caledon Deer Park, PA42, Woodstock, PU31, (Alexander & Anderson 2012); **Italy:** Sicily, UTM(?) (Wallin *et al.* 2009), Bibione, UL45 (Gutowski *et al.* 2010), Brixen, QS07, Fennberg, PS62, Mitterberg, UTM(?), Südtirol, UTM(?) (Hellrigl *et al.* 2012); **Latvia:** Maļinova, MC80, Moricsala Isl., EJ63-64, Saulkrasti, LD44, Šedere, MB49 (Barševskis & Savenkov 2013); **Netherlands:** Kraggenburg, FU93-94 (Cuppen 2010); **Norway:** Rygge: Telemarkslunden, NL98, Oslo: Oslo, NM94, Vestby: Son, NL99, Ås: Ås, PM01, Drøbak, NM91, Oppegård, PM02, Frogn: Blylaget, NM92, Larvik: Pauler, NL54, Løveskogen, NL64, Stokke: Melsomvik, Brunstad, NL76, Hof: Sæteråsen, NM60, Nøtterøy: Østre Bolærne, NL86, Drangedal: Drangeland, NL05,Skultrevassåsen, NL14, Kragerø: Kragerø, NL22, Grønåsliane, NL12, Skien: Skien, NL36, Ulvskollen, NL26, Arendal: Arendal, MK88, Tromøy, MK98, Risør: Risør, NL10, Laget, Rangleåsen, NL00, Froland: Froland, MK78, Ripåsen, MK89, Gjerstad: Gjerstad, NL02, Vardeheia, ML93, Grimstad: Reddalsvann, MK66, Kristiansand: Stangenes, MK44, Grostøl, Karlsmoen, MK45, Bråvann, MK34, Farsund: Straumen, LK64 (Wallin et al. 2009, Kvamme & Wallin 2013, 2016); **Poland:** Barania Góra Mt., CV59, Barwinek, EV47, Białe Błota, XU98, Białowieża Primeval Forest, FD94, Biebrzański NP, FE01, Borecka Forest, Lipowo, EE79, Bukowa Forest, Kołowo, VV70, Drozdowo, ED79, Grobla Chonczarowska, FE00, Hańsk Drugi, FB69, Jankowice, XU11, Kacice, ED03, Karpacz, WS52, Kopna Góra, FE60, Kościelec, CC38, Kraków-Przegorzały, DA14, Krynica, DV97, Łódź, CC94, Marianów, Grady, EC21, Miszek, CD55, Młodów, DV77, Niepołomicka Forest, Ispina, DA55, Ojców NP, DA16, Paleśnica, Ruda Kameralna, DA81, Promno, XU51, Przemyśl-Prałkowce, FA21, Puszczykówko, Jeziory, XT29, Radocyna Mt., EV27, Romincka Forest, FF01, Sieraków res., Warszawa-Młociny, DC99, Skawce, CA91, Sycyn Dolny, XU13, Tunel, DA28, Zakopane, DV26, Żegiestów, DV87, Ziemięcice, CA38, (Gutowski *et al*. 2010), Jaworzno, CB35 (Karpiński *et al.* 2011), Mików, EV86 (Kurzawa *et al*. 2012), Pińczów, DA79 (Plewa *et al*. 2014b), Kampinoski NP, Kalisko, DC79 (Górski & Tatur-Dytkowski 2015), Dobrynia, EV29, Wysowa Zdrój, EV17 (Karpiński *et al.* 2015), Rogów, DC24 (Borowski & Sławski 2016); **Russian Federation:** the Curonian Spit, DG80, Kaliningrad, DF66, Zelenogradsk, DF69 (Alekseev & Bukejs 2011); **Serbia:** Rtanj, EP74 (Ilić & Ćurčić 2013), Belgrade, DQ55, Guberevac Forest, DQ53, Kazan-Ploče, FQ03, Kupinovo, DQ25, Ljig, DP39-49, Miroč, FQ02, Svilajnac, EP19, Tekija, FQ04, Tresibaba Mt., EP91, Vršac, EQ29 (Ilić & Ćurčić 2015); **Sweden:** Gotland, UTM(?) **(**Wallin *et al*. 2009); **Ukraine:** Halych, LQ34, Hodyni, FA52, Lomachyntsi, NQ22, Lviv, KR82, Ternopil, LQ98 (Gutowski *et al*. 2010), Brzuchowice, GA13, Bucyki, MQ26, Ivano-Frankove, FA93, Klishkivtsi, P46, Krutyliv, MQ45, "Maslyatyn", near vlg. Dibrova, MQ(?), near city of Lviv, GA12, Novosilky, LR31, Roztochya res., FA93, Stavky, FA93, Stradch, FA93, Synkiv, MP28, Tovste, MQ01, Vikno Forestry, MQ36, "Vovchnetski Hory", FA93, Vyshniv, LQ16, Yavoriv (Zalissya) UTM(?) (Zamoroka *et al*. 2012), Charnushvychi, UTM(?), Dora, LP27, Humnyska, LR33, Kiwerce, LS93, Mohyliv-Podilsky, NP56, Tukhlya, FV82, Uzhgorod, EU98, "Vutva" ad Pischa, FC(?), Vynohradiv, FU53, Yamna, LP26, Zrub, LQ15 (Zamoroka & Kapelyukh 2012).

Wing measurements. Hind wings of *L. linnei* (Fig. 1a) and *L. nebulosus* (Fig. 1b) were dissected from the body, straightened and mounted between two microscopic slides. Subsequently, wing images (with 4800 dpi resolution) were obtained using an Epson V330 Photo scanner (Suwa, Japan). On each image 15 homologous points (landmarks) (Fig. 1c) were determined manually using DrawWing software (Tofilski 2004).

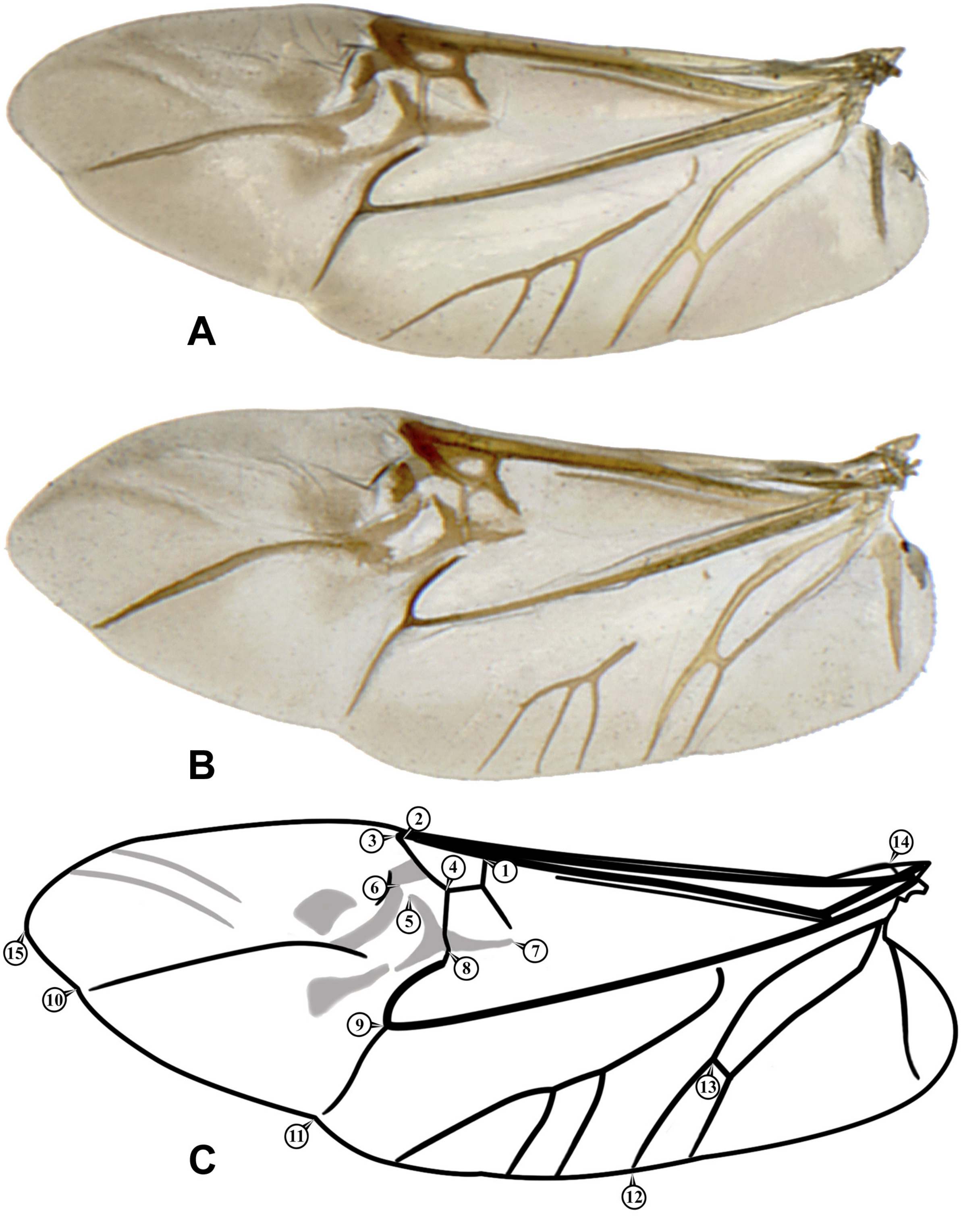

FIGURE 1. The hind wings of *Leiopus linnei* (A) and *L. nebulosus* (B). The wings were measured by obtaining coordinates of 15 landmarks (C). The vein ending at landmark 10 is named RP2 (Kukalová-Peck & Lawrence 2004).

Statistical analysis. Prior to the analysis, the coordinates of the landmarks were aligned using generalized orthogonal least-squares procedures (Rohlf & Slice 1990). The procedure involves scaling, translation and rotation of the landmarks. After the alignment coordinates of homologues landmarks can be compared. The wings shape was described with partial warp scores obtained using tpsRelw (1.54 v.) software (Rohlf 2010) and the wing size was assessed as centroid size. Centroid size was calculated by taking the square root of the sum of squared distances between each landmark and the centroid of each wing. The canonical variate analysis (CVA) of wing shape and size was used for species discrimination. Identification models were validated using leave-one-out method (jackknife) in PAST 3.11 software (Hammer *et al.* 2001). The identification algorithm was exported to IdentiFly software (Przybyłowicz *et al.* 2015) which is freely available at www.drawwing.org/identifly. The analysis of variance (ANOVA) was used to analyze the differences between the two species in wing size. The multiple analysis of covariance (MANCOVA) with wing size as covariate was conducted to estimate the potential effect of allometry on wing shape of *Leiopus* beetles. For visualization of the wing shape difference MorphJ (version 1.06a) was used (Klingenberg 2011).

Results

Wings morphometrics. The wing size differed significantly between *L. linnei* and *L. nebulosus* (ANOVA: $F_{1,156} = 37.38$, $p < 0.001$; Fig. 2). Sexual dimorphism in wings size was also significant (ANOVA: $F_{1,156} = 4.13$, $p < 0.043$). In both species females had larger wings than males. Interaction between the two factors (species and sex) was not significant (ANOVA: $F_{1,156} = 0.71$, $p > 0.412$).

A MANCOVA revealed that there is allometric relationship between wing shape and size (Table 1). Significant interaction between factors of species and size indicates that the allometric relationship differed between species (Table 1). The wing shape differed significantly between species but not between sexes (Table 1). There were some differences in wing shape between males and females, however, they can be explained by allometry.

TABLE 1. MANCOVA of hind wing shape in *Leiopus* species. Significant effects ($p \leq 0.05$) are in bold.

	Wilks Λ	*F*	Effect df	Error df	*P*
species	0.70	2.07	26	127	**0.004**
sex	0.85	0.89	26	127	0.620
size	0.47	5.56	26	127	**0.000**
species*sex	0.79	1.26	26	127	0.200
species*size	0.70	2.09	26	127	**0.004**
sex*size	0.85	0.88	26	127	0.628
species*sex*size	0.79	1.26	26	127	0.199

The wing measurements allowed visualizing differences in hind wing shape between *L. linnei* and *L. nebulosus* (Fig. 3). The main difference was position of folding line (between landmarks 3 and 11) in relation to wing base (landmark 14). In *L. linnei* the folding line was further away from the wing base. Moreover, radial cell (landmarks 1–4) in *L. linnei* was smaller, and end of vein RP2 (wing venation terminology follows Kukalová-Peck & Lawrence (2004)) was closer to the wing tip. Apical field in *L. linnei* is also narrower and the distance between landmarks 3 and 11 is smaller than in *L. nebulosus*. It should be noted, however, that those differences are difficult to discern without measurements.

CVA of wing shape allowed to clearly separate the two species (Fig. 4). There were, however, some outliers. Two females of *L. nebulosus* occurred together with *L. linnei*. Spermathecae of the two specimens were intermediate between the two species. It is possible that the two females were misidentified. Some overlap between the convex hulls in Fig. 4 is result of presenting three dimensional space on two dimensional plot.

If data for males and females were combined identification based on all measurements of wing shape allowed to correctly classify (with cross-validation) 97.39% in *L. linnei* and 95.56% in *L. nebulosus*. Only 5 of all 160 examined specimens of *Leiopus* sp. were misidentified, which gives 96.88% identification correctness. Analysis of males separately allowed to correctly classify all of them. In case of females the correct classification rate was 93.8%.

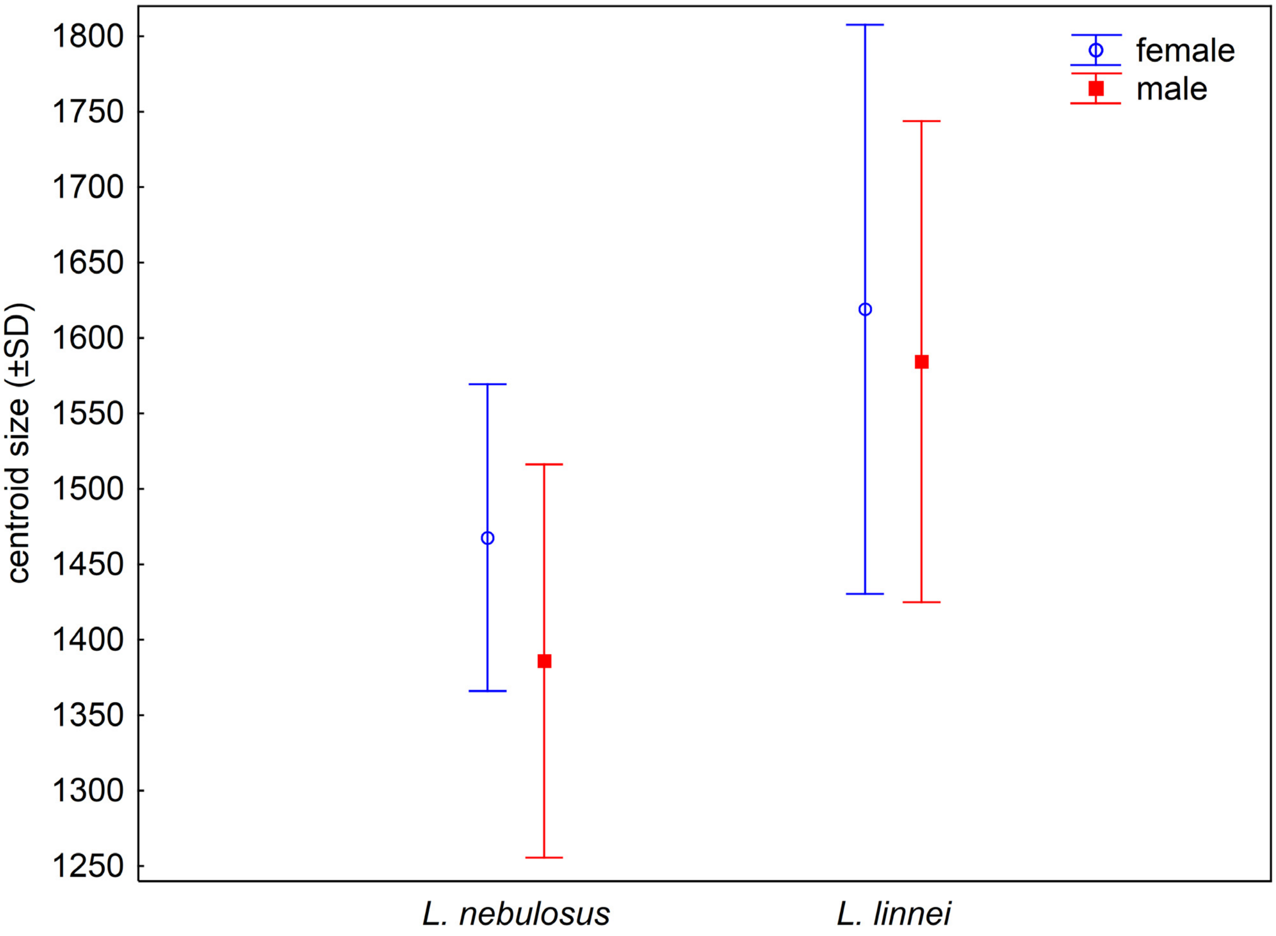

FIGURE 2. Differences in hind wing size between *Leiopus linnei* and *L. nebulosus.*

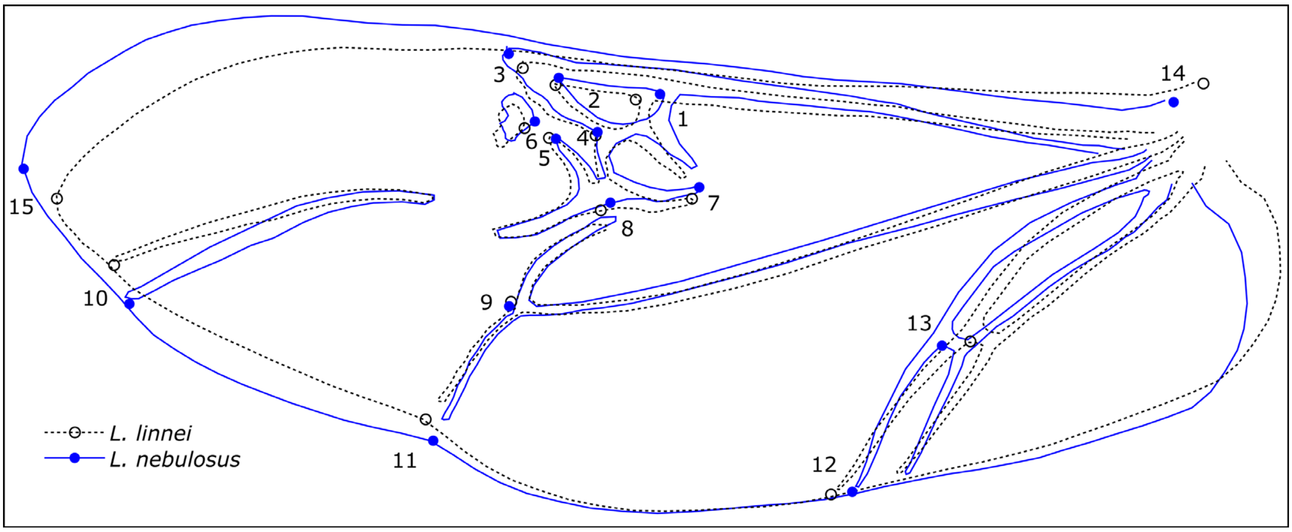

FIGURE 3. Differences in hind wing shape between *Leiopus linnei* and *L. nebulosus*. Differences were exaggerated 3 times to make them more visible. The position of the lines is a result of interpolation which is less accurate at greater distance from the landmarks.

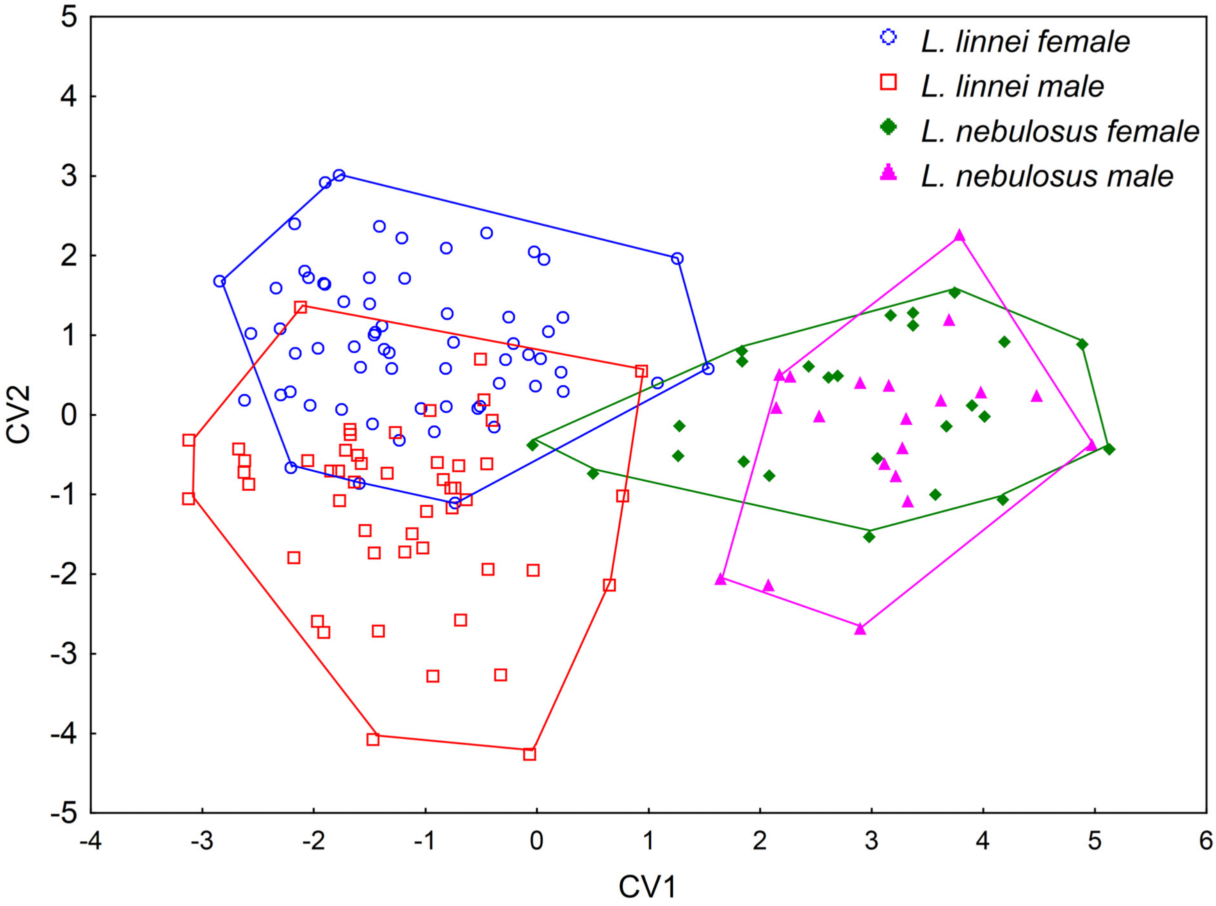

FIGURE 4. Discrimination of species and sexes of *Leiopus* based on canonical variate analysis. Lines indicate convex hulls.

In order to simplify the identification only two distances between the landmarks can be measured. The distances differed markedly between the species. The first distance between landmarks 1 and 14 (referred as distance 1–14) was 3.98±0.47 and 3.42±0.32 mm (mean±SD), in *L. linnei* and *L. nebulosus*, respectively. The second distance between landmarks 10 and 15 (referred as distance 10–15) was 0.83±0.11 and 0.92±0.10 mm, in *L. linnei* and *L. nebulosus*, respectively. CVA based on the two distances allowed to correctly identify 87.1% of individuals. Canonical variates coefficients were used to obtain simple identification index *i*:

$i = 2.33\ d_{1\text{-}14} – 8.27\ d_{10\text{-}15}$

where $d_{1\text{-}14}$ and $d_{10\text{-}15}$ are distances 1–14 and 10–15, respectively. The distances need to be expressed in millimeters. If the index *i* is greater than 1.37 a specimen should be classified as *L. linnei* otherwise is should be classified as *L. nebulosus*.

Species distribution ranges. The two species in the *L. nebulosus* species group are European and West-Palearctic species. The group (before splitting), was recorded in most European countries (Sama 2002; Danilevsky 2012) (Fig. 5a, 5b, area delimited by solid line). The northern limit runs somewhere around 60°45' of northern latitude (Kvamme & Wallin 2013). The eastern border is the Ural mountain range which the group crosses only in its southern part where it somewhat invades the Asian continent (in the western regions of Kazakhstan). To the south, the Caucasus is another barrier for this group of species (they are known from Armenia, Azerbaijan and Georgia) (Danilevsky 2012). The limit of distribution proceeds further via the Crimea, northern Turkey and southern Greece, to Sicily, and Corsica (Sama 2002; Danilevsky 2012). The western limit goes across northern part of Spain and Portugal (Vives 2000; Sama 2002).

These species are also found in Ireland and Great Britain (Wallin *et al.* 2009; Alexander & Anderson 2012). The known occurrences of both species overlap over the majority of the range. The significant differences appear in the number of localities. After 2009, the occurrence of *L. linnei* has been confirmed in 19 countries (Fig. 5a) and *L. nebulosus* in 15 countries (Fig. 5b). *Leiopus linnei* seems to be more widely distributed and a more frequently collected species.

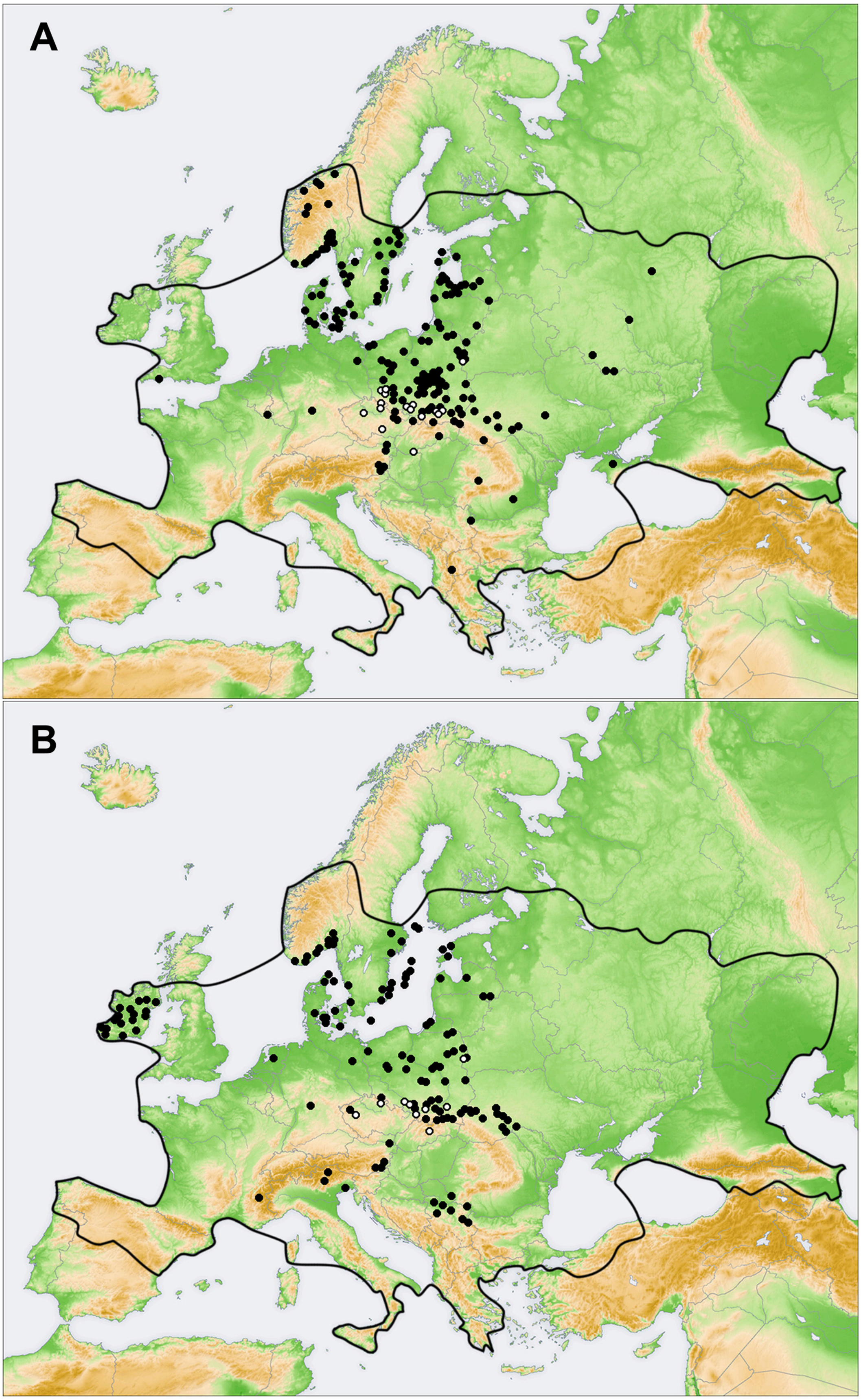

FIGURE 5. The map of Europe with the distribution range of *Leiopus linnei* (A) and *L. nebulosus* (B). Black line indicates distribution of *L. nebulosus* species group before splitting into two species. The points show localities: filled circles are records from literature and open circles are based on examined specimens.

Discussion

We have documented here new morphological characters which can be used to separate *L. nebulosus* and *L. linnei*. The original separation of the two species was well supported by both genetic and morphological investigations (Wallin *et al*. 2009). However, the species identification is difficult (Mikát & Hamet 2014). The results presented here indicate possibility of identification of *L. nebulosus* and *L. linnei* on the basis of hind wings measurements. The method of landmark-based geometric morphometrics has been successfully used to separate many groups of sibling species and subspecies, particularly among Hymenoptera and Diptera (Oleksa & Tofilski 2014), both ecotypes (Combey *et al.* 2013), and sibling species (Baylac *et al.* 2003; Villemant *et al.* 2007). The method can also be effectively applied in identification of beetles (Su *et al.* 2015; Rossa *et al.* 2016, Goczał *et al.* 2016). The characters used in traditional identification of insects are often subjective. More precise morphometric measurements permit much better efficiency in identification of species (Mutanen & Pretorius 2007). Furthermore, it allows expressing differences between species in objective, quantitative terms. However, the most important aspect is to provide a method of identifying insects by less experienced entomologists. The only requirement for effective use of this method is to have some basic knowledge of insect morphology.

Only a few steps are required to successfully identify a *Leiopus* specimen by using the presented here method. After the hind wing preparation, which can be successfully conducted even by person with low experience in entomology (Goczał *et al.* 2016), wing image should be obtained by using a desktop scanner. Subsequently, wing images should be loaded to the IdentiFly software, and the 15 landmarks (Fig. 1c) should be placed manually by mouse clicking. After that, the software determines the probability that the specimen belongs to each of the *Leiopus* species. The low probability levels indicate that the specimen belongs to another species, or that the preparation is inappropriate. It is also possible to use simplified method involving measurements of only two distances. The distances can be measured without computer, for example, using ocular micrometer. However, in case of simplified method the accuracy of identification is much lower.

Error of identification based on wing measurements is relatively low even if only one wing is used, however, it should be noted that various environmental factors may affect insect wing shape and size. Therefore, it is recommended to measure both the left and the right wing. If available, several specimens from each population should be measured. In order to obtain even higher certainty, the identification should be confirmed by inspection of the traditional characters. In general males should be preferred because they are identified more successfully.

The results so far show that the distribution of *L. linnei* is wider than that of *L. nebulosus*. Further, *L. nebulosus* has not been reported from eastern Europe (Fig. 5b), and its possible presence there was questioned (Telnov *et al.* 2010; Danilevsky 2012; Egorov & Ruchin 2013; Ruchin 2015). Future faunistic studies may fill the gaps. The number of localities and specimens caught or observed shows that *L. linnei* should be considered as a common species. *Leiopus nebulosus* was reported from many European localities after 2009. However, several authors emphasize the small size of local populations (Ilić & Ćurčić 2013; Borowski & Sławski 2016).

Another interesting but unexplained issue is the origin of the species' sympatry and the extent of niche overlap or competition avoidance. Both species are polyphagous and their host trees preferences overlap largely. As many as 22 genera of host trees has been documented for *L. linnei*: *Acer*, *Aesculus*, *Alnus*, *Carpinus*, *Corylus, Fagus*, *Juglans, Malus*, *Padus*, *Picea*, *Pinus*, *Populus*, *Quercus*, *Rhus*, *Salix*, *Sorbus*, *Ulmus* (Gutowski *et al.* 2010), *Tilia* (Telnov *et al.* 2010), *Prunus*, *Robinia* (Górski & Tatur-Dytkowski 2015), *Betula*, *Frangula* (Borowski & Sławski 2016). *Leiopus nebulosus* has been recorded from 16 genera: *Alnus*, *Corylus*, *Quercus*, *Padus*, *Picea*, *Tilia* (Gutowski *et al*. 2010), *Ficus, Fagus, Prunus* (Wallin *et al.* 2009), *Malus* (Alekseev & Bukejs 2011), *Carpinus* (Karpiñski *et al.* 2011), *Castanea*, *Cerasus*, *Salix* (Hellrigl *et al.* 2012), *Populus* (Górski & Tatur-Dytkowski 2015), *Juglans* (examined material). Despite large similarity in host species, some differences may be found in substrate conditions preferred by the two species. There are some evidences that *L. linnei* tends to prefer wood with relatively higher moisture content than *L. linnei* (Wallin *et al.* 2009), whereas *L. nebulosus* seems to prefer warmer and drier conditions (Kvamme & Wallin 2013). Some differences may be also found in host tree diameter. It was showed that *L. linnei* inhabits relatively thicker branches and stems than *L. nebulosus* (Gutowski *et al.* 2010; Kvamme & Wallin 2013). Differences in host preferences can be found also between other pair of sympatric species from Lamiinae subfamily: *Stenostola dubia* (Laicharting, 1784) and *S. ferrea* (Schrank, 1776) (Wallin *et al.* 2005; Kvamme & Wallin 2011).

The taxonomic impediment issue is perceived as one of the greatest problems faced by studies on biological diversity (Fattorini *et al.* 2012; Coelman 2015). Even in such a popular and well-studied group of insects as Cerambycidae there are still a great number of taxa of unclear taxonomic status (Sama 2002) which often are difficult to identify even by experienced entomologists. This may results in limited knowledge of their distribution, population size as well as of their ecology. We regard further development of identification process of particularly troublesome groups of species is therefore of utmost importance.

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