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The species status of the *Otiorhynchus clavipes* (Bonsdorff, 1785) species group (Coleoptera: Curculionidae): an integrative approach using molecular, morphological, ecological, and biogeographical data

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Abstract. The species of the *Otiorhynchus clavipes* (Bonsdorff, 1785) group (Coleoptera: Curculionidae) treated here, are characterized by their unusually high phenotypical variation, which often caused taxonomic problems and controversies. Molecular markers COI and EF1-α, karyological analysis, as well as morphological, biogeographical and ecological data are used to study weevils collected in the Alps, Carpathians, Sudetes and different areas of Germany. In the investigated populations of the flightless species *O. fagi* Gyllenhal, 1834 and *O. clavipes*, we detected an interspecific genetic distance of 11.3–15.8% (COI) and 3.1–3.7% (EF1-α) depending on geographical distance. The phylogenetic trees indicate that both species are monophyletic and that they were correctly delimited from each other. Both species have also separate geographical ranges in Central Europe. Male specimens differ in the morphology of the aedeagus and the last abdominal sternite. Our study supports the legitimacy of species delimitation of *O. fagi* and *O. clavipes* as separate species, which can be treated as stable taxonomic hypotheses. The determination of the species status required the re-examination of species ranges and allowed together with data on biology and altitudinal preferences a better biogeographical and ecological characterization of the species.

Keywords. Weevils, *Otiorhynchus*, integrative taxonomy, species delimitation, molecular markers (COI, EF1-α), biodiversity, distribution.

INTRODUCTION

Inconsistent definitions and taxonomic treatment of cryptic species prevent informed estimates of their contribution to biodiversity and impede our understanding of their evolutionary, biogeographical and ecological significance (Struck et al. 2018). The cryptic concept refers to two or more species that have been classified as a single nominal species including subspecies and varieties due to the fact that they are apparently morphologically very similar, show subtle regional or ecological adaptations and/or are completely indistinguishable (Zúniga-Reinoso & Benítez 2015). Careful taxon-specific approaches and knowledge are consequently required to discriminate between species. One of the most common suppositions is that most cryptic species result from speciation phenomena that are so recent that morphological traits or any other traditional diagnostic characters have not yet evolved (Winker 2005). In this sense, the hyperdiverse genus *Otiorhynchus* Germar, 1822, which current systematics is based mainly on the morphological structure of adults, includes many cryptic species which have yet to be discovered.

The genus *Otiorhynchus* (Curculionidae: Entiminae: Otiorhynchini) is the most species-rich of all weevil genera in Europe and the West Palaearctic Region, comprising around 1500 valid species and 105 subgenera within the main distribution area in the Palaearctic region (Hoffmann 1950; Smreczyński 1966; Dieckmann 1980; Knutelski 2005; Germann 2010; Stüben et al. 2015; Mazur 2016). In Europe, the tribe Otiorhynchini includes eight genera, and the subgenus *Otiorhynchus* Germar, 1822 sensu stricto is represented by 64 species (Alonso-Zarazaga et al. 2017). The highest numbers of species of the genus *Otiorhynchus* occur in the European mountains (Dieckmann 1980), and many local forms inhabit the high montane and alpine regions and especially areas

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with non-glaciated refugia and nunataks. *Otiorhynchus* species occurring north of the Alps, the Sudetes and Carpathians (including the Tatra Mts.) represent only a very small part of the species of this speciose genus (e.g., Hoffmann 1950; Smreczyński 1966; Magnano 1998; Knutelski 2005; Germann 2010; Stüben et al. 2015; Mazur 2016). *Otiorhynchus* species, which are without exception incapable of flight, are known as general feeders with a large number of host plant genera comprising deciduous and coniferous trees, woody plants, perennial dicots and perennial grasses (Smreczyński 1966; Koch 1992; Knutelski 2005; Sprick & Stüben 2012). Adults are leaf feeders and cause characteristic bite marks on host plant leaves, and the larvae are subterraneous, ectophagous root feeders (Willis 1964; Dieckmann 1980; Gosik et al. 2016).

One of the most problematic taxa arousing much controversy among many current taxonomists are the species related to *Otiorhynchus fagi*, previously called *Otiorhynchus tenebricosus* (Herbst, 1784) group (Casalini & Colonnelli 2019), which probably includes two or more cryptic species with a distribution restricted to certain areas inside the range. This phenotypically extremely variable taxon was interpreted differently in the past, which led to the description of many subspecies, varieties and forms, and the creation of a huge number of species synonym names.

In the opinion of Hoffmann (1950), *Otiorhynchus lugdunensis* Boheman, 1843 together with *O. hungaricus* Germar, 1824, *O. fuscipes* (Olivier, 1807) and *O. clavipes* (Bonsdorff, 1785) form a complex of closely related but separate species in Western Europe. Other authors considered them forms of one variable species. For example, Lona (1936) distinguished within *O. clavipes* three taxa as varieties, and Hoffmann (1950) regarded these as subspecies and subdivided additionally each subspecies into varieties. In turn, Frieser (1981) included in the *O. clavipes* group: *O. lugdunensis*, *O. fuscipes, O. clavipes* sensu stricto, and *O. hungaricus*. Morris (1997) treated *O. lugdunensis* and *O. fuscipes* in Great Britain as synonyms of *O. clavipes*, but he explained that the true status is unresolved and needs further study especially on biology. Smreczyński (1966) distinguished *O. fuscipes* and *O. lugdunensis* from Poland and other countries of Eastern Europe using morphological features and he listed them as separate species. Likewise, Dieckmann (1980) treated both taxa as separate and valid species in eastern Germany. In his opinion, some specimens from the mountain and the low mountain ranges, determined as *O. clavipes,* belong either to *O. fuscipes* or to *O. lugdunensis*, and *O. hungaricus* was regarded as a southeastern European species with an isolated occurrence in southern France. The status of *O. hungaricus* Germar, 1824 as separate species from other taxa of this group raised the least controversy and is widely accepted (e.g., Endrödi 1961; Mazur 2002; Alonso-Zarazaga et al. 2017; Yunakov et al. 2018). Currently, Casalini & Colonnelli (2019) synonymized the French subspecies *Otiorhynchus hungaricus hospitellensis* Hustache, 1923 with the nominotypical taxon. Magnano (2001) synonymized *O. clavipes* (Bonsdorff, 1785), *O. fuscipes* (Olivier, 1807), and *O. lugdunensis* Boheman, 1843 together with more than 30 taxa to a very diverse species cluster (Delbol 2010, 2013; Alonso-Zarazaga 2014) under the name of *O. tenebricosus* (Herbst, 1784). Several other authors (e.g., Germann 2010; Löbl & Smetana 2011) adopted this proposal at first. Wanat & Mokrzycki (2005) also accepted the synonymization of *O. lugdunensis* but with some objections, arguing ecological distinctness from the nominotypical *O. tenebricosus*. However, some researchers using different methods studied and indicated the dissimilarity between *O. tenebricosus* and *O. lugdunensis*. Germann (2013) reinstalled *O. lugdunensis* as a valid species from synonymy with *O. tenebricosus* based on differences in the morphological structure of the aedeagus of both taxa. Gosik & Sprick (2013) studied the morphological structure of pupae and found some diagnostic features specific to each of both taxa. Further evidence for the status of *O. lugdunensis* as separate species came from the p-distance (COI) of 11.4-11.6% from *O. tenebricosus* (Schütte et al. 2013; Stüben et al. 2015). Probably based on these results, the name *O. lugdunensis* was used again as a separate taxon in the catalogues of Löbl & Smetana (2013) and Alonso-Zarazaga et al. (2017). However, in those two catalogues the synonymy of *O. clavipes* and *O. fuscipes* with the species *O. tenebricosus* remained unchanged. The study of the larval morphology of the *O. tenebricosus* group (Gosik et al. 2016) confirmed the validity of *O. lugdunensis* as reported by Schütte et al. (2013); Germann (2013); and Stüben et al. (2015) against synonymisation suggested by Magnano (2001) and Fauna Europaea (Alonso-Zarazaga 2014). Wanat & Mokrzycki (2018) also withdrew *O. lugdunensis* from synonymy with *O. tenebricosus*, although the relation to the very similar *O. clavipes* remained unresolved. Casalini & Colonnelli (2019) designated a neotype for *Curculio clavipes* Bonsdorff, 1785, rediscovered several overlooked synonymies and discovered new synonyms of this species. Recently, *O. tenebricosus* was synonymized with *O. fagi* by Casalini & Colonnelli (2019).

Therefore, it was necessary to verify all available data and explore this species group more in detail except the well-defined *O. hungaricus*. The ecological differences between the forms, particularly in Central Europe and the United Kingdom, were also taken into account, which may provide additional evidence to shed light on this problem. Thus, the aims of this paper are: 1) to compare morphology, karyology and the results of molecular analyses of adult weevils of the taxa *Otiorhynchus clavipes* (Bonsdorff, 1785), *O. fuscipes* (Olivier, 1807), and *O. lugdunensis* Boheman, 1843, collected in the Alps, Carpathians, Sudetes and different areas of Germany; 2) to find diagnostic characters useful to distinguish these taxa, 3) to report new bionomic data, and 4) to present an updated distribution of these taxa.

MATERIAL AND METHODS

A total of 66 adult specimens were collected for this study from 16 localities in the Alps (Slovenia), Carpathians (Poland and Slovakia), Sudetes (Poland) and different regions in Germany, using sweep nets, beating trays or direct collecting from plants and under stones, over a period of ten years (2009–2018). A full list of localities and their coordinates, biotopes, and collecting plants with these sites is given in Table 1. Additional specimens from various regions of Germany, England and France were used for morphological descriptions and photos of habitus and abdominal sternites (Figs 7–8). All individuals were a priori identified to species level using the keys of Smreczyński (1966) or Dieckmann (1980). Nomenclature of scientific names follows Alonso-Zarazaga et al. (2017); Wanat & Mokrzycki (2018); and the PolBIN (2019).

The gonads of 12 alive individuals (males) of each taxon were dissected for chromosome analysis, the other 45 individuals were preserved in 99.8% ethanol and stored at -20°C for molecular and morphometric studies.

Information on morphology, ecology and biogeography of the studied taxa is based on the authors' own data and Hoffmann (1950, 1963); Smreczyński (1966); Endrödi (1961); Knutelski (2005); Sprick & Stüben (2012); Germann (2013); Mazur (2016); and Gosik et al. (2016).

Molecular analysis

DNA was isolated from complete insects. In order to avoid the damage of body parts which were later used in morphometry the specimen were poked with a needle on the abdomen to facilitate the flow of genetic material. NucleoSpin tissue kit (Macherey-Nagel) with suitable procedure was used for the isolation process. Two DNA fragments, mitochondrial cytochrome oxidase 1 (COI) and nuclear elongation factor α (EF1-α), were amplified with PCR technique in a reaction mix consisting of: 3 µl of DNA, 2 µl of buffer x10 with $MgCl_2$, 0.6 µl of dNTPs, 0.6 µl of primer F, 0.6 µl of primer R, 0.2 µl of Taq polymerase, 13 µl of water. Amplification was conducted with the use of the following primers: EF-F sequence (5' AGAACGTGAACGTGGTATCA 3'), EF-R sequence (5' CTTGGAGTCACCAGCTACATAACC 3'), LCO1490-JJ sequence (5' CHACWAAYCATAAAGATATYGG 3') HCO2198-JJ sequence (5' AWACTTCVGGRTGVCCAAARAATCA 3') (Astrin & Stüben 2008; Hernandez-Vera et al. 2013).

The amplification was performed in a Mastercycler EpigradientS (Eppendorf) with the following profile: 95°C for 4 min, 95°C for 30 s, 50°C for COI and 55°C for EF1, for 1 min, 72°C for 2 min followed by 35 cycles at 95°C for 30 s, 50°C for 1 min, 72°C for 2 minutes and a final extension step at 72°C for 10 min and 1 min at 10°C. The effectiveness of the amplification was checked by conducting electrophoresis in 1% agarose gel tinted with Midori Green Advance DNA Stain (NIPPON Genetics) (100 mg/ml). Purified PCR products were sequenced with Big Dye Terminator v3.1. Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions with the same primers that were used in the PCR reaction. Sequencing reaction was conducted by Genomed S.A., Warsaw.

Alignment and phylogenetic analysis

Sequences were read and edited using BioEdit v.7.2.6 (Hall 1999) and then aligned using ClustalX (Thompson et al. 1997). The most suitable model of nucleotide substitution was determined by using MrModeltest 2.3 (Nylander 2004) in conjunction with PAUP*4.0b (Swofford 2002). The GTR+G model was chosen for COI (proportion of invariable sites I = 0, gamma distribution shape parameter G = 0.2521), the GTR model for EF-1α (proportion of invariable sites I = 0, equal rates for all sites).

We used two methods to determine phylogeny: Bayesian inference (BI) and maximum likelihood (ML). BI was ran using MrBayes 3.1 (Ronquist & Huelsenbeck 2003; Nylander et al. 2004) with one cold and three heated Markov chains for 3,000,000 generations and trees were sampled every 100th generation. Each simulation was run twice. Stationarity was considered to be reached when the average standard deviation of split frequencies was <0.01; the convergence of each run was visually inspected using Tracer v. 1.5.0 (Rambaut et al. 2009), and appropriate number of sampled trees were discarded as 'burn-in', and a majority-rule consensus tree was obtained. Maximum likelihood was run using RAxML v. 8.0.0 (Stamatakis 2014) with a bootstrap resampling of 100 replicates via the rapid bootstrap procedure of Stamatakis et al. (2008) to assign support to branches in the ML tree.

Genetic distances were computed in PAUP*4.0b (Swofford 2002) by using uncorrected "p" model. Each tree has one outgroup – *Liparus glabirostris* Küster, 1849 (for EF1 marker) or *Liophloeus tessulatus* (O.F. Müller, 1776) (for COI marker), both species are from the same family (Curculionidae). All trees were visualized with TreeView 1.6.6 (Page 1996).

Chromosomes

The gonads of 12 individuals (males) of each species were dissected and used as material for slides. The gonads were fixed in Carnoy (3:1 96% ethanol: glacial acetic acid). The squashes were performed on dry ice in

Table 1. Sampling areas, locations, date, biotopes and plants of collected weevils used in molecular analysis: Germany, Poland, Slovakia and Slovenia. Abbreviations: m a.s.l. = meter above sea level, N = individual numbers, Leg. = name of collector, APC = a priori categorisations, C = *Otiorhynchus clavipes*, L = *O. lugdunensis*, T = *O. tenebricosus*.

Sampling area	Sampling site	Geograph. coordinates	Altitude (m a.s.l.)	Date	Biotope	Plants	N	Leg.	APC	Remarks
				Germany						
Lower Saxony (LSH)	Hannover-Linden, park south of Leine river	N52°22′40″ E09°42′30″	53	2011-04-06, 2017-03-23, 2017-04-12	park	*Euonymus japonica*, *E. fortunei*, *Ligustrum vulgare*, *Fraxinus* spec.	4	Sprick	L	rather abundant, very local in a small area (< 100 square meters); beating in the late evening and early night
Lower Saxony (LSB)	Bad Zwischenahn-Aschhausen	N53°12′18,5″ E08°04′22,5″	12	2012-07-09, 2013-07-02	tree nursery	*Thuja occidentalis*	2	Sprick	L	rare, collected at night
Lower Saxony (LSL)	Ith Mountains (Lauenstein)	N52°04′35″ E09°32′56″	350–400	2012-03-26			5	Sprick	T	abundant
Lower Saxony	National Park Harz: Harz Mts., at Oderteich lake, spruce forest	N51°46′16″ E10°32′03″	740	2012-07-02	coniferous forest, mountain forest	*Picea abies*, *Vaccinium myrtillus*	11	Sprick	T	abundant, beaten from plants and collected under stones
Schleswig-Holstein (SHP)	Pinneberg: Rellingen, hedgerow	N53°38′25″ E09°50′22″	10	2013-06-04	tree nursery	*Thuja plicata* ’*Aurescens*’	3	Sprick	L	rare, beating in the afternoon
Schleswig-Holstein (SHB)	Bullenkuhlen, hedgerow	N53°46′24″ E09°45′04″	8	2011-05-11, 2013-06-04	tree nursery	*Thuja* spec. *cf. plicata*	10	Sprick	L	rather rare; mainly females
North Rhine-Westphalia (NRW)	Paderborn, Wewer, Ziegenberg, forest edge	N51°41′13″ E08°42′43″	140-150	2012-07-15	hillside forest on calcareous ground	*Fraxinus excelsior*, *Euonymus europaea*, *Cornus sanguinea*	4	Sprick	C	not rare, beaten at daytime from the lower parts of bushes and young trees
Baden-Württemberg (BWH)	Heilbronn						1	Sprick	L	

Table 1. Continued.

Sampling area	Sampling site	Geograph. coordinates	Altitude (m a.s.l.)	Date	Biotope	Plants	N	Leg.	APC	Remarks
					Poland					
Carpathians (CTM)	Tatra Mountains: Nad Piecem	N49°14′59″ E19°53′13″	1500	2011-06-26	glade	*Picea abies, Vaccinium myrtillus*	4	Knutelski	T	beating from plants at the edge of a glade
Carpathians (CGM)	Gorce Mts.: Kiczora	N49°32′24″ E20°08′59″	1240	2012-06-20	spruce forest	*Picea abies*	5	Knutelski	T	beating
Carpathians (CZB)	Zywiec Beskids Mts.: Sucha Góra	N49°31′38″ E19°07′52″	675	2011-06-03	spruce forest	*Picea abies*	1	Knutelski	T	beating
Sudetes (SKM)	Karkonosze Mts.: Karpacz Górny	N50°77′12″ E15°71′87″	950	2013-07-05	spruce forest	*Picea abies*	7	Knutelski	T	beating from trees in the lower montane belt
Sudetes (SSM)	Stolowe Mts. Pasterka	N50°29′15″ E16°19′39″	740	2009-08-04	spruce forest		2	Mazur	T	
					Slovakia					
Carpathians	Low Tatras Mts.: Liptovska Luzna	N48°56′24″ E19°22′03″	950	2013-06-10	spruce forest	*Picea abies*	1	Knutelski	T	beating
					Slovenia					
Alps (AKM)	Karawank Mts.: Părlicevo sedlo	N46°27′40″ E14°00′30″	1125	2013-06-07	spruce forest	*Picea abies, Vaccinium myrtillus*	2	Knutelski	T	beating at mountain pass near Austrian border in Jesenice community

Table 2. Measured body parts of the studied taxa for morphometric analysis.

Body parts	Measurements
head	width width of rostrum eye diameter distance between eyes distance: eye-basis of antennae
elytra	width length
pronotum	width length
metasternum	width of left and right part
sternum	width length
aedeagus	length of dorsal view width of dorsal view length of lateral view width of lateral view angle of apex
receptaculum seminis	width height

70% acetic acid. The finished preparations were dried with warm air and stained in 4% Giemsa phosphate buffer (pH 6.8) for 10 min. Well-spread spermatogonial metaphases were selected for the determination of chromosome numbers. Observations of chromosomes and photomicrographs were made using a Nikon Eclipse 50i microscope.

Morphological measurements

Weevils were dissected, mounted on separate mounting cardboards and labelled. Males and females from the same locality were identified as the same taxon. Measurements of twenty morphometric characters were analyzed in this study (Table 2). Additionally, sex dimorphistic features were also measured (five for males and two for females). A Nikon SMZ1500 stereomicroscope, with NIS Elements software, was used for all morphometric measurements. All body parts were photographed and measured. Every measurement was taken five times and averaged.

Statistical analyses of all morphometric data were conducted using R software version 3.4.1 (r-project.org) and slightly modified versions of the R-scripts provided by Baur & Leuenberger (2011). Scatterplots were generated with the package 'ggplot2' (Wickham 2009). Missing measurements were replaced by mean average of respective body parts. All data were standardized by transforming into a logarithmic scale.

Additionally, bl: body length (from front margin of the eyes to apex of elytra/body), bw: body width (largest width of body), hw: head width, were measured of four taxa specimens whose habitus is shown in Fig. 7, as well as the width and length of the five abdominal sternites (AS) of the five taxa presented in Fig. 8.

Majority of the specimens used in this study are deposited in the Department of Entomology at Institute of Zoology and Biomedical Research, Jagiellonian University of Krakow (Poland), and those presented in Figs 7–8 are in the collection of P. Sprick.

RESULTS

DNA analysis

In the case of COI marker analysis, the biggest genetic distance (15.8%) was detected between *Otiorhynchus fagi* from the Alps: Karawank Mountains (Slovenia) and *Otiorhynchus clavipes* from Schleswig-Holstein: Bullenkuhlen (Germany). Compared with other populations of *O. clavipes* in Germany the genetic distance is similar (15.6%). In turn, genetic distance between six populations of *O. clavipes* in Germany and five populations of *O. fagi* in the Carpathians (Poland), and two populations in the Sudetes (Poland) is almost similar (11.3–11.6%). Only in one population of *O. fagi* from the Gorce Mts. (Carpathians) there is a bigger genetic distance (12.5%) to *O. clavipes* (Table 3).

The results of EF1-α marker analysis of five *O. clavipes* populations from Germany and six *O. fagi* populations from Poland (four from the Carpathians and two from the Sudetes) show the biggest genetic distance (4.7%) between *O. fagi* from the Carpathians: Zywiec Beskids (Poland) and *O. clavipes* from Schleswig-Holstein: Bullenkuhlen (Germany). The genetic distance between the *O. lugdunensis* population from Bullenkuhlen and *O. fagi* populations from the Carpathians and the Sudetes is a bit lower (in each case 4.2%). However, the genetic distance of EF1-α between the other five *O. clavipes* populations from Germany and five *O. fagi* populations from the Polish Carpathians and the Sudetes is clearly smaller (mean value 3.1%) (Table 4).

Using two phylogenetic methods and two different markers (mitochondrial and nuclear), trees of similar topology were obtained (Figs 1–2). On each tree *O. fagi* and *O. clavipes* form separate clades, which implies that they are correctly divided. *Otiorhynchus clavipes* is monophyletic in both trees. These results also indicate the relatively high genetic variation of *O. fagi*, especially for COI

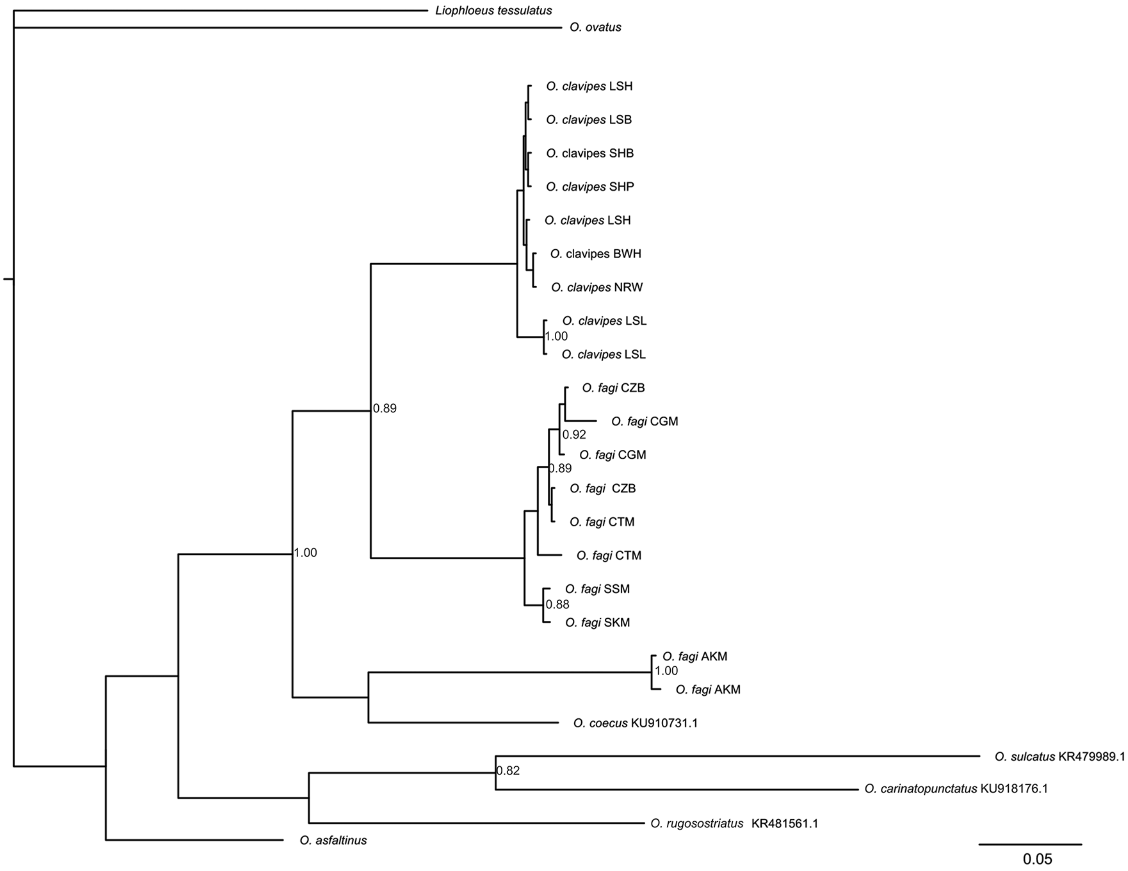


Fig. 1. Bayesian approach consensus tree for COI data. Numbers at nodes indicate Bayesian posterior probability: LSB = Lower Saxony: Bad Zwischenahn, LSH = Lower Saxony: Hannover; SHB = Schleswig-Holstein: Bullenkuhlen; SHP = Schleswig-Holstein: Pinneberg; NRW = North Rhine Westphalia; BWH = Baden-Wuerttemberg: Heilbronn (GERMANY); CZB = Carpathians: Zywiec Beskid Mts.; CGM = Carpathians: Gorce Mts.; CTM = Carpathians: Tatra Mts.; SSM = Sudetes: Stolowe Mts.; SKM = Sudetes: Karkonosze Mts. (POLAND); AKM = Alps: Karawank Mts. (SLOVENIA). Sequences from *O. ovatus*, *O. coecus*, *O. sulcatus*, *O. carinatopunctatus*, *O. rugosostriatus* downloaded from GenBank.

data (Fig. 1). These COI data also show that Alpine populations are notably different from the Carpathians and Sudetes populations, and in the Carpathians genetic differences between populations within individual mountain ranges are present (Table 3, Figs 1–2). In the case of *O. clavipes* genetic differences between populations were relatively smaller than in the previous species (Fig. 1). The EF1-α data confirm genetic differences of *O. fagi* in the Carpathians and indicate a small variability of some German *O. clavipes* populations (Figs 3–4), however, we had unfortunately no data for the populations from the Karawank Mts. (Slovenian Alps).

Chromosomes

Otiorhynchus fagi and *O. clavipes* share the same diploid complement of 22 chromosomes and a sex determination mechanism of the parachute type (Xy_p). The meioformula $n = 10+Xy_p$ was identical at all metaphase I plates of spermatid division (Figs 5–6).

Morphometric differences

Before the analysis, data on body size were normalized and then analyzed with principal component analysis (PCA). This analysis was performed to evaluate body size correlations between two species among males and females. We focused on PCs with eigenvalues >1 (Quinn and Keough, 2002), following our PCA of the body size data, we considered three principal components, PC1, PC2 and PC3. To test differences between both species we used general linear model (GLM). Amid males and females we observed no significant differences for all PC's (Tables 6, 7). The Principal component analysis (PCA) showed no statistically significant differences between *Otiorhynchus fagi* (T) and *O. clavipes* (L), both for females (Fig. 7) and males (Fig. 8).

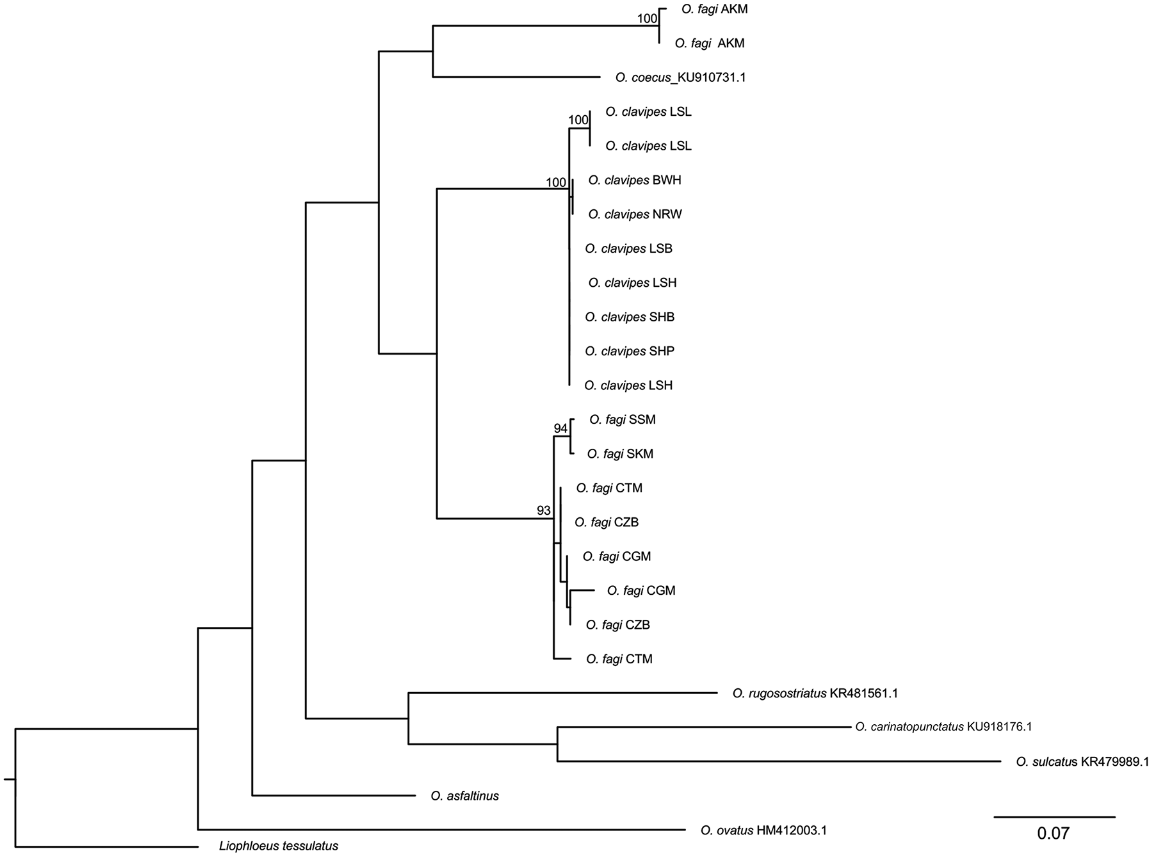


Fig. 2. Maximum-likelihood tree for COI data. Numbers at nodes indicate bootstrap values: LSB = Lower Saxony: Bad Zwischenahn, LSH = Lower Saxony: Hannover; SHB = Schleswig-Holstein: Bullenkuhlen; SHP = Schleswig-Holstein: Pinneberg; NRW = North Rhine Westphalia; BWH = Baden-Wuerttemberg: Heilbronn (GERMANY); CZB = Carpathians: Zywiec Beskid Mts.; CGM = Carpathians: Gorce Mts.; CTM = Carpathians: Tatra Mts.; SSM = Sudetes: Stolowe Mts.; SKM = Sudetes: Karkonosze Mts. (POLAND); AKM = Alps: Karawank Mts. (SLOVENIA). Sequences from *O. ovatus*, *O. coecus*, *O. sulcatus*, *O. carinatopunctatus*, *O. rugosostriatus* downloaded from GenBank.

Additional information on species

The main morphological features to distinguish *O. fagi* and *O. clavipes* are the sculpture and pubescence of the upper surface of the body as well as of the underside, the length of the antennal scrobe and of the funicle, the coloration of the legs, the different body shape and especially the number of grooves of the last abdominal sternite (AS) in the male sex (Figs 9–10). The females of both taxa are marginally different (see Table 5).

Both taxa occur in Central Europe and form two extremes among other representatives of the genus *Otiorhynchus* regarding these characteristics and molecular differences demonstrated earlier (Tables 1–2), and they behave in this area in terms of their ecology and biogeography as two separate species (Table 5).

Based mainly on own observations of the authors, the description of the species has been supplemented with information from the publications mainly of Hoffmann (1950); Smreczyński (1966); Dieckmann (1980); Germann (2013); and other authors mentioned in the text.

Otiorhynchus fagi Gyllenhal, 1834
(Figs 9–10, Table 5)

Synonyms were presented according to the order of data descriptions of species names, after Alonso-Zarazaga et al. (2017) and Casalini & Colonnelli (2019):

= *Curculio clavipes* Bonsdorff, 1785: 40
= *Curculio rufipes* Sturm, 1792: pl. 17
= *Curculio tenebricosus* Herbst, 1795: 333 not *Curculio tenebricosus* Herbst, 1784
= *Curculio haematopus* Schrank, 1798: 490 not *Curculio haematopus* Gmelin, 1790, replacement name for *Curculio tenebricosus* Herbst, 1795
= *Otiorhynchus erythropus* Boheman, 1842: 267
= *Curculio fuscipes* Olivier, 1807: 372 not *Curculio fuscipes* Geoffroy in Fourcroy, 1785

Table 3. Genetic distance for CO1 marker: SHB = Schleswig-Holstein: Bullenkuhlen; LSH = Lower Saxony: Hannover; LSB = Lower Saxony: Bad Zwischenahn; NRW = North Rhine Westphalia: Paderborn; SHP = Schleswig-Holstein: Pinneberg (GERMANY); CTM = Carpathians: Tatra Mts.; CZB = Carpathians: Zywiec Beskid Mts.; CGM = Carpathians: Gorce Mts.; SSM = Sudetes: Stolowe Mts.; SKM = Sudetes: Karkonosze Mts. (POLAND); AKM = Alps: Karawank Mts. (SLOVENIA).

Taxa/no	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**	**11.**	**12.**	**13.**	**14.**	**15.**	**16.**
1. *O. fagi* CTM	–															
2. *O. fagi* CZB	0.00000	–														
3. *O. fagi* CGM	0.00344	0.00344	–													
4. *O. fagi* CZB	0.00515	0.00515	0.00344	–												
5. *O. fagi* CGM	0.01546	0.01546	0.01375	0.01375	–											
6. *O. fagi* SSM	0.02234	0.02234	0.02577	0.02405	0.03093	–										
7. *O. fagi* SKM	0.02234	0.02234	0.02577	0.02405	0.03093	0.00344	–									
8. *O. fagi* CTM	0.01890	0.01890	0.02062	0.02234	0.02921	0.02405	0.02405	–								
9. *O. clavipes* SHB	0.11512	0.11512	0.11512	0.11512	0.12543	0.11684	0.11684	0.11340	–							
10. *O. clavipes* LSH	0.11512	0.11512	0.11512	0.11512	0.12543	0.11684	0.11684	0.11340	0.00172	–						
11. *O. clavipes* LSB	0.11512	0.11512	0.11512	0.11512	0.12543	0.11684	0.11684	0.11340	0.00172	0.00000	–					
12. *O. clavipes* NRW	0.11512	0.11512	0.11512	0.11512	0.12543	0.11684	0.11684	0.11340	0.00172	0.00000	0.00000	–				
13. *O. clavipes* SHP	0.11512	0.11512	0.11512	0.11512	0.12543	0.11684	0.11684	0.11340	0.00172	0.00000	0.00000	0.00000	–			
14. *O. fagi* AKM	0.14777	0.14777	0.14605	0.14605	0.14948	0.15120	0.15120	0.14605	0.15464	0.15292	0.15292	0.15292	0.15292	–		
15. *O. fagi* AKM	0.15120	0.15120	0.14948	0.14948	0.15292	0.15464	0.15464	0.14948	0.15808	0.15636	0.15636	0.15636	0.15636	0.00344	–	
16. *L. tessulatus* OUTGROUP	0.18051	0.18051	0.18041	0.17844	0.18075	0.19217	0.19220	0.18801	0.19491	0.19298	0.19298	0.19298	0.19298	0.18860	0.19065	–

Table 4. Genetic distances for EF1α marker: LSB = Lower Saxony: Bad Zwischenahn; LSH = Lower Saxony: Hannover; NRW = North Rhine Westphalia: Paderborn; SHP = Schleswig-Holstein: Pinneberg; SHB = Schleswig-Holstein: Bullenkuhlen (GERMANY); CTM = Carpathians: Tatra Mts.; SSM = Sudetes: Stolowe Mts.; SKM = Sudetes: Karkonosze Mts.; CGM = Carpathians: Gorce Mts.; CZB = Carpathians: Zywiec Beskid Mts. (POLAND).

Taxa/no	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**	**10.**	**11.**	**12.**
1. *O. fagi* CTM	–											
2. *O. fagi* SSM	0.00000	–										
3. *O. fagi* CTM	0.00000	0.00000	–									
4. *O. fagi* SKM	0.00000	0.00000	0.00000	–								
5. *O. fagi* CGM	0.00000	0.00000	0.00000	0.00000	–							
6. *O. fagi* CZB	0.00524	0.00524	0.00524	0.00524	0.00524	–						
7. *O. clavipes* LSB	0.03147	0.03147	0.03147	0.03147	0.03149	0.03671	–					
8. *O. clavipes* LSH	0.03147	0.03147	0.03147	0.03147	0.03149	0.03671	0.00000	–				
9. *O. clavipes* NRW	0.03147	0.03147	0.03147	0.03147	0.03149	0.03671	0.00000	0.00000	–			
10. *O. clavipes* SHP	0.03147	0.03147	0.03147	0.03147	0.03149	0.03671	0.00000	0.00000	0.00000	–		
11. *O. clavipes* SHB	0.04196	0.04196	0.04196	0.04196	0.04198	0.04720	0.01049	0.01049	0.01049	0.01049	–	
12. *L. glabrirostris* OUTGROUP	0.23741	0.23741	0.23741	0.23741	0.23681	0.23961	0.24619	0.24619	0.24619	0.24619	0.25030	–

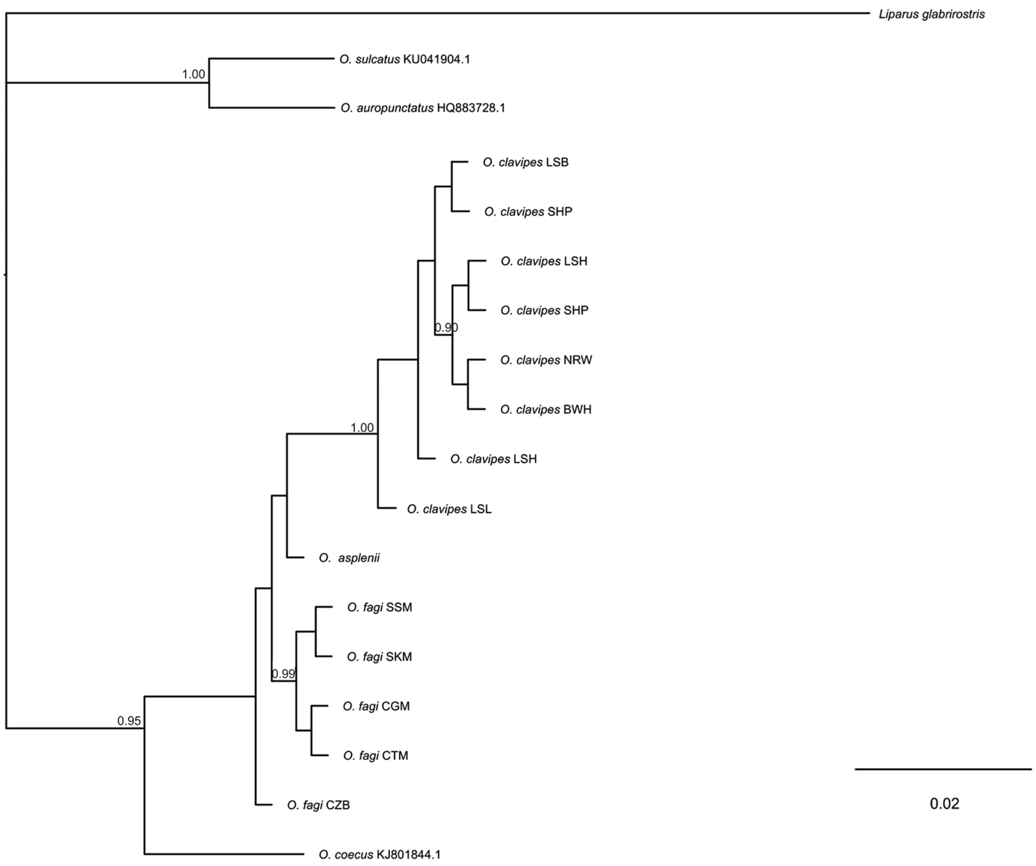


Fig. 3. Bayesian approach consensus tree for EF1-α data. Numbers at nodes indicate Bayesian posterior probability: LSB = Lower Saxony: Bad Zwischenahn, LSH = Lower Saxony: Hannover; SHP = Schleswig-Holstein: Pinneberg; NRW = North Rhine Westphalia; BWH = Baden-Wuerttemberg: Heilbronn (GERMANY); CZB = Carpathians: Zywiec Beskid Mts.; CGM = Carpathians: Gorce Mts.; CTM = Carpathians: Tatra Mts.; SSM = Sudetes: Stolowe Mts.; SKM = Sudetes: Karkonosze Mts. (POLAND). Sequences from *O. sulcatus*, *O. auropunctatus*, *O. coecus* downloaded from GenBank.

= *Curculio maritimus* Donovan, 1811: 63
= *Otiorhynchus substriatus* Silbermann, 1833: no. 7
= *Otiorhynchus substriatus* Gyllenhal, 1834b: 563
= *Otiorhynchus sanguinipes* Boheman, 1842: 296
= *Otiorhynchus waltoni* Smith, 1869: 136
= *Otiorrhynchus sanguinipes* var. *subglaber* Reitter, 1913: 50
= *Otiorhynchus fuscipes* forma *heynei* Voss, 1919: 405
= *Otiorhynchus evertsi* Uyttenboogaart, 1931: 292
= *Otiorrhynchus sanguinipes* var. *stierlini* Uyttenboogaart, 1931: 295 not *Otiorhynchus stierlini* Gemminger, 1871
= *Otiorrhynchus stierlinianus* Uyttenboogaart, 1933: 229 not *Otiorrhynchus populeti* Boheman, 1842 var. *stierlinianus* Reitter, 1914b: 159, replacement name for *Otiorrhynchus sanguinipes* var. *stierlini* Uyttenboogaart, 1931
= *Otiorhynchus olivieri* Abbazzi & Osella, 1992: 294, replacement name for *Curculio fuscipes* Olivier, 1807 not *Curculio fuscipes* Geoffroy in Fourcroy, 1785

Adult morphology. *Otiorhynchus fagi* is quite variable, depending on the origin, such as in the coloration of the legs (knees and base of femorae more or less darkened but not as clear as in *O. coecus*), in the fine pubescence of the top of the body and especially in the sculptures and spaces of the elytral striae. Generally, *O. fagi* is morphologically similar to *O. clavipes*, but it is weaker and narrower in habit than the usually large, strong form of *O. clavipes*. The body length is 8–13 mm. The antennae are black. The upper side of the pronotum is unevenly scored in the front part, in the back part it is equipped with small granules, just like on the sides. The sides of the pronotum are rarely pubescent and hardly stand out from the upper part. On the elytra there are very small and very sparse patches of scales. Along the elytral striae small punctures are clearly visible. The tarsi are black. Body hairs are rare and very delicate, so that the beetle appears bare; exceptionally hair clusters at the punctures of grooves merging into very small spots on the sides of elytra are hardly noticeable.

Table 5. Additional distinctness in morphology, ecology and biogeography between *Otiorhynchus fagi* Gyllenhal, 1834 and *O. clavipes* (Bonsdorff, 1785; compilation of the authors' own data and information from Hoffmann (1950, 1963), Smreczyński (1966), Endrödi (1961), Knutelski (2005), Sprick & Stüben (2012), Germann (2013), Mazur (2016) and Gosik et al. (2016); for illustrations see Figs 7–8.

Feature/Species	*O. fagi* Gyllenhal, 1834	*O. clavipes* (Bonsdorff, 1785)
Morphology		
body shape	weaker and narrower than *O. clavipes*; no bright spots on elytra, antennae black, small points clearly visible on the elytral striae	stronger and broader than *O. fagi*; head black with a cherry stripe behind the eyes, white hair spots on elytra, femorae cherry
size	9–13 mm	9.5–15 mm
elytra	very small and very sparse, scaly patches present	usually loosely covered by groups of small silver-grey spots
abdominal sternite	only very fine grooves in front of the apex, less expanded and less deep, in great number (more than 40), no crest of yellowish hairs or bristles at the apex	about 8–15 strong ribs in the middle, accompanied by a different number of small ribs/grooves at the sides and a crest of yellow hairs/bristles at the top border
aedeagus	apex rounded, the inner bag with several complex tortuous sclerites	apex usually angular, the inner bag almost completely reduced
larva coloration	head dark brown; all thoracic and abdominal segments from dark yellow to brownish	head yellow or dark yellow; all thoracic and abdominal segments yellow or yellowish to brownish
Ecology		
location	mainly in mountainous and piedmonts areas	in western Europe in lowlands and areas of low and medium height (low mountain ranges), introduced in central and eastern Europe mainly in urban estates in sandy regions
habitat	mainly in forest areas, deciduous and mixed, from the lower to the high mountain regions; abundant in different habitats in the Sudetes and Carpathians Mts.	in great parts of Western Europe in the forest zone of the low hill country; in introduced areas (e.g. the northern plains) mainly in allotments, nurseries, parks, thickets, cemeteries, hedges, and gardens
food	adults feed leaves of various plants, such as European spruce, also of herbaceous perennials, producing characteristic feeding notches	adults feed leaves and buds mainly of common lilac and privet, also recorded from other Oleaceae plants and adjacently growing bushes, producing characteristic feeding notches similarly to *O. fagi*
appearance of adults	May–October, and most often in June and July; nocturnal, in the mountains also during the day	April–September, most abundant in April–May; mainly nocturnal
Biogeography		
geographic distribution	most mountainous regions of Central and Eastern Europe, in the hill country of Germany, in the Alps, Sudetes and Carpathians	mainly in western and central Europe, introduced in eastern Europe and very rare and local in this area; increasing its range with the planting of common lilac (*Syringa*), white-cedar (*Thuja*) and privet (*Ligustrum*)
altitudinal distribution	400–2500 m a.s.l., mostly abundant and frequent in 800-2100 m a.s.l.	up to 200 m a.s.l. (in France also found in some higher mountain areas)
biogeographical element	European, mountain	European, mountain and lowland

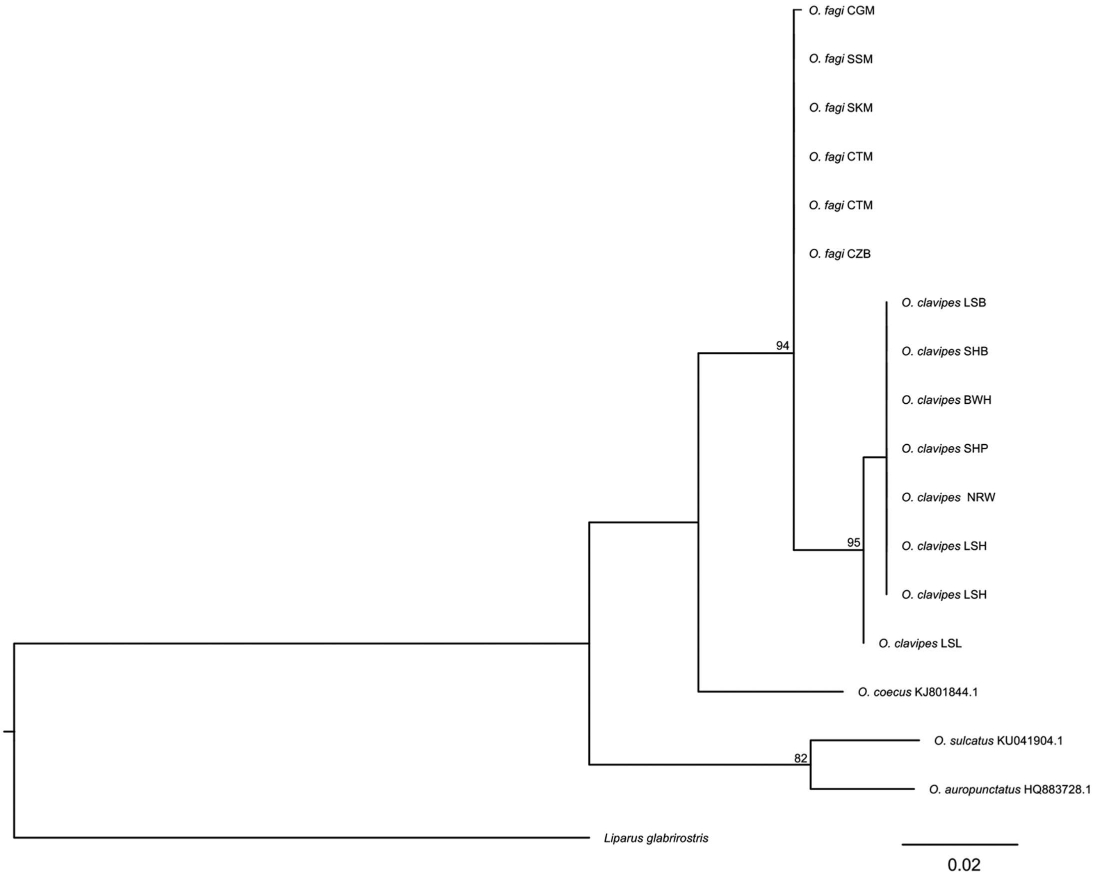


Fig. 4. Maximum-likelihood tree for EF1-α data. Numbers at nodes indicate bootstrap values: LSB = Lower Saxony: Bad Zwischenahn, LSH = Lower Saxony: Hannover; SHB = Schleswig-Holstein: Bullenkuhlen; SHP = Schleswig-Holstein: Pinneberg; NRW = North Rhine Westphalia; BWH = Baden-Wuerttemberg: Heilbronn (GERMANY); CZB = Carpathians: Zywiec Beskid Mts.; CGM = Carpathians: Gorce Mts.; CTM = Carpathians: Tatra Mts.; SSM = Sudetes: Stolowe Mts.; SKM = Sudetes: Karkonosze Mts. (POLAND). Sequences from *O. sulcatus*, *O. auropunctatus*, *O. coecus* downloaded from GenBank.

Male. The elytra are elongate. The last abdominal sternite (AS) is flat and characterized by a great number (> 40) of very fine grooves in front of the apex, less expanded and less deep, bearing no crest of yellowish hairs or bristles at the posterior edge of the apex (Fig. 8). The AS of *O. fagi* is the smallest (1.75 × 1.175 mm = 1.49: 1) among the compared AS of all measured taxa (Fig. 8). In appearance it is very similar to AS of *O. coecus* Germar, 1823, used here as the „outgroup". The aedeagus of *O. fagi* is almost parallel laterally in the apical region, and the apex is rounded, the inner sac is armed with several complex tortuous sclerites (Germann 2011, 2013; Schütte et al. 2013).

Female. The last abdominal sternite has very fine furrows in the central part, and it bears fine hairs at the sides and at the apex as on the other sternites.

Larva. The head is dark brown; all thoracic and abdominal segments from dark are yellow to brownish; cuticle is almost smooth. For more details on the morphology of the larva see the publication of Gosik et al. (2016).

Ecology. This species inhabits mainly montane and submontane areas, from the deciduous and mixed forests of the middle and higher low mountain regions to the coniferous forest of higher elevations. In the Tatra Mts. *O. fagi* mainly inhabits spruce forests, fir-spruce forests, mountain pine, subalpine and alpine meadows, rowan-spruce and willow-rowan brushwood, but it is also found in beeches, alders, in brush and herbs at roadsides, at streams and at windbreak, on clearings as well as in couloirs and on turfs on the rocks. *Otiorhynchus fagi* does not show clear habitat preferences and can be classified as eurytopic weevil species of the hill and mountain regions (Hoffmann 1950; Smreczyński 1966; Dieckmann 1980; Morris 1997; Knutelski 2005; Sprick & Stüben 2012; Stüben et al. 2015; Gosik et al. 2016; Mazur 2016).

Food plants. Polyphagous, adults feed on leaves of various plants, both woody and herbaceous, making characteristic feeding notches on *Picea abies* (L.) H. Karst., *Abies alba* Mill., *Pinus mugo* Turra, *Alnus incana* (L.) Moench, *Corylus avellana* L., *Salix* spp., *Sorbus*

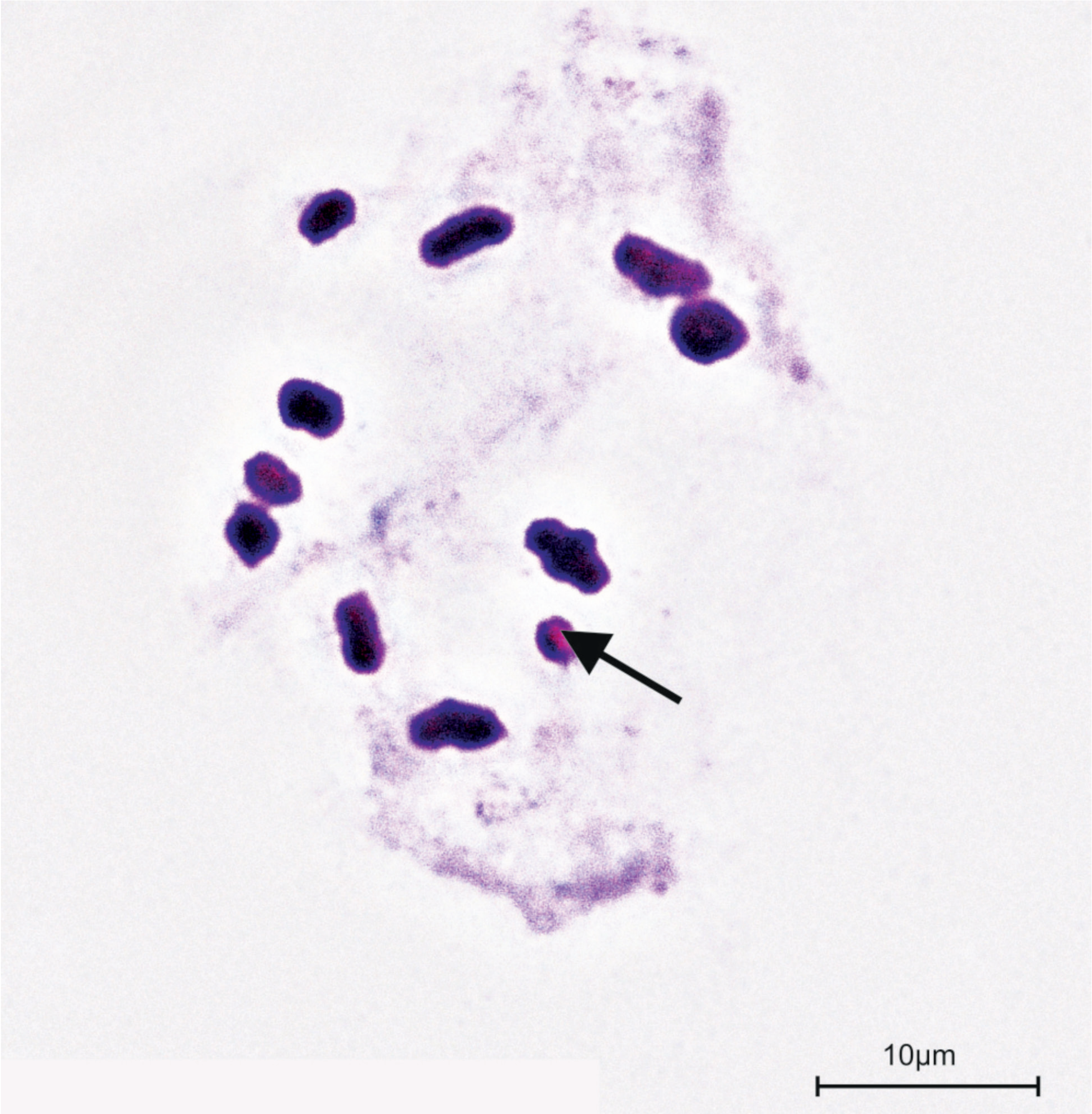


Fig. 5. Chromosomes of *Otiorhynchus fagi*, arrow indicates Xyp (the sex chromosomes of parachute type).

aucuparia L., *Rubus idaeus* L., *Petasites albus* Gaertn., *Adenostyles alliariae* A. Kern., *Rumex acetosa* L., *Trifolium pratense* L., *Vaccinium myrtillus* L., various species of *Alchemilla, Cirsium* and *Geranium*; in the Carpathians and Harz Mts. collected mainly from *Picea abies* (Hoffmann 1950; Dieckmann 1980; Palm 1996; Knutelski 2005; Stüben et al. 2015; Kizub and Slutsky 2019; P. Sprick, pers. data). In the Czech Republic the species was collected also from *Rosa* sp., and from *Solanum tuberosum* L. at agricultural potato crops areas near a forest (Hrabovský 2014).

Appearance of adults. From beginning of May to October, and most often in June and July; on and under different plants, under stones and in the leaf litter (Dieckmann 1980; Knutelski 2005; Gosik et al. 2016).

Geographical distribution. This species occurs in several central and east European mountainous regions, from Eastern France to Romania.

In Western Europe additionally present in the Massif Central, and possibly, but very isolated and to confirm in the French Pyrenees (two sites) and in adjacent regions of Northern Spain (two to three sites). In the Apennines of northern and central Italy and on Monte Amiata (see Casalini & Colonnelli 2019), a volcano of Pleistocene origin in Tuscany, it was also recorded from the northern Mediterranean region. In Germany occurring in all regions of the Alps and Central Uplands with northern

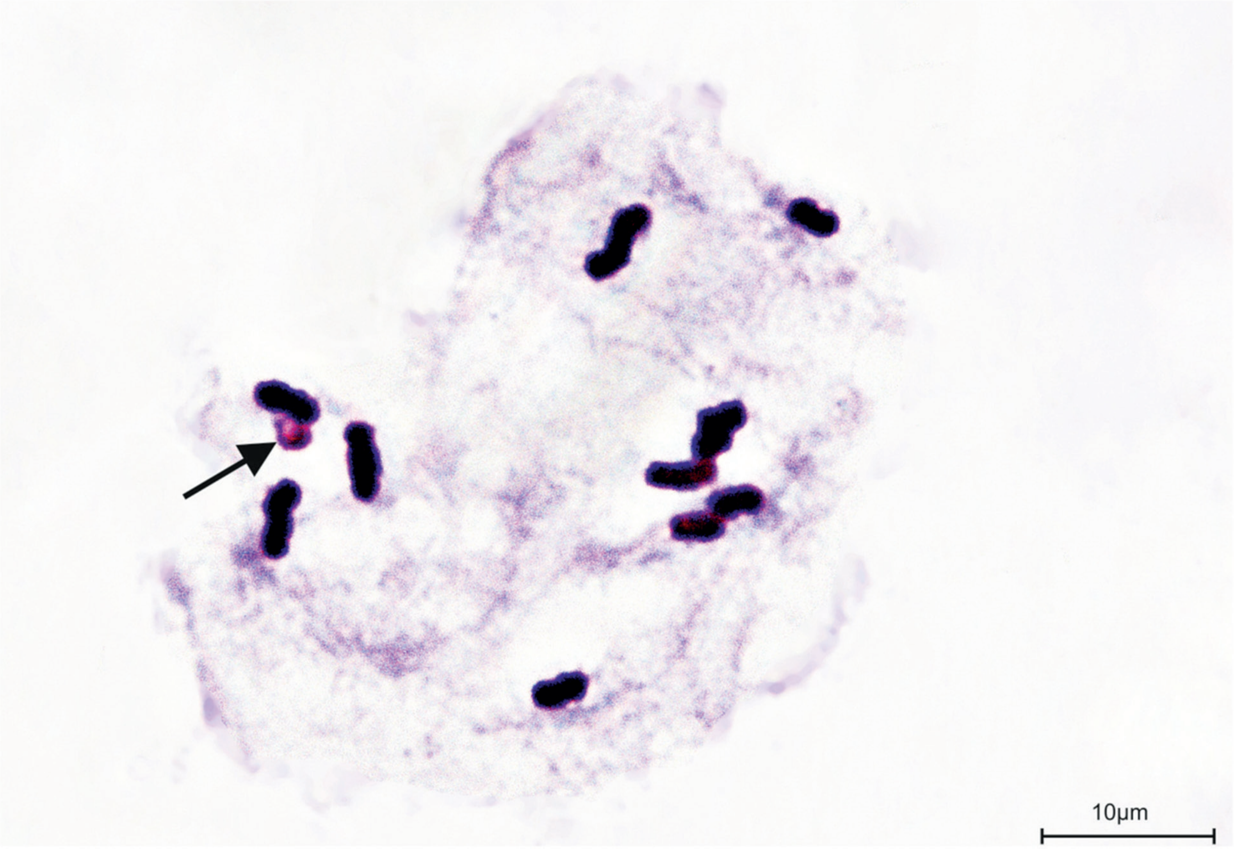


Fig. 6. Chromosomes of *Otiorhynchus clavipes*, arrow indicates Xyp (the sex chromosomes of parachute type).

limit in the Ith and Harz Mountains and absent or nearly absent from the northern sandy plains (Köhler & Klausnitzer 1998; Bleich et al. 2020).

On the Balkan Peninsula only present in the northern or northwestern part (Croatia).

In the Sudetes and the Carpathian areas of Poland the species is common, and in particular it is widespread and often very frequent in the Tatra Mts., as well as in the Slovakian, Ukrainian and Romanian Carpathians (Knutelski 2005; Kizub & Slutsky 2019; own data of P. Sprick and S. Knutelski). It is widespread and quite frequent in Switzerland: Jura Mts., midlands, north side of the Alps, Western and Eastern Central Alps (Germann 2010; in conjunction with Germann 2013; and C. Germann, pers. comm. 2020).

It is widespread also in the southeastern part of France: Alpes de Savoie, Alpes du Dauphiné, Hautes Alpes, Rhône-Alpes (Isère, Ain), Massif Central/Auvergne (Cantal, le Lioran, le Sancy, Puy-de-Dôme), Vaucluse (Mt. Ventoux), and Vosges (Hoffmann 1950; Schott 2017).

In Hungary is known only from several localities (northern part, in the environs of the Bükk highland, close to the frontier with Slovakia) (Slieker 2019), present also in Banat (Western Romania) and in Croatia, where it is rare (Endrödi 1961). Also known from many places in hillside areas of the same regions.

In Poland the largest enclave outside mountain regions is located in the Kraków-Wielun Upland; it includes the Prądnik valley in the Ojców National Park.

It was recorded once or sporadically in a few localities in the highlands and lowlands, too (Hoffmann 1950; Endrödi 1961; Smreczyński 1966; Dieckmann 1980; Knutelski 2005; Germann 2013; Sprick & Stüben 2012; Stüben et al. 2015; Mazur 2016; Yunakov et al. 2018; Kizub and Slutsky 2019).

According to Alonso-Zarazaga et al. (2017) and new information by Casalini and Colonnelli (2019), our own data, information from some colleagues and further research, the revised distribution is as follows: Austria, Belgium, Croatia, Czech Republic, France, Germany, Hungary (northern part), Italy (northern to central part) (Abbazzi & Maggini 2009; R. Casalini, Roma, pers. comm. 2020), Liechtenstein, Luxembourg, Poland (southern part), Romania, Slovakia, Slovenia, ?Spain, Switzerland, and Ukraine (western part). Information on introduction of *O. tenebricosus* to North America by Alonso-Zarazaga et al. (2017) probably refers to *O. clavipes* (see next break).

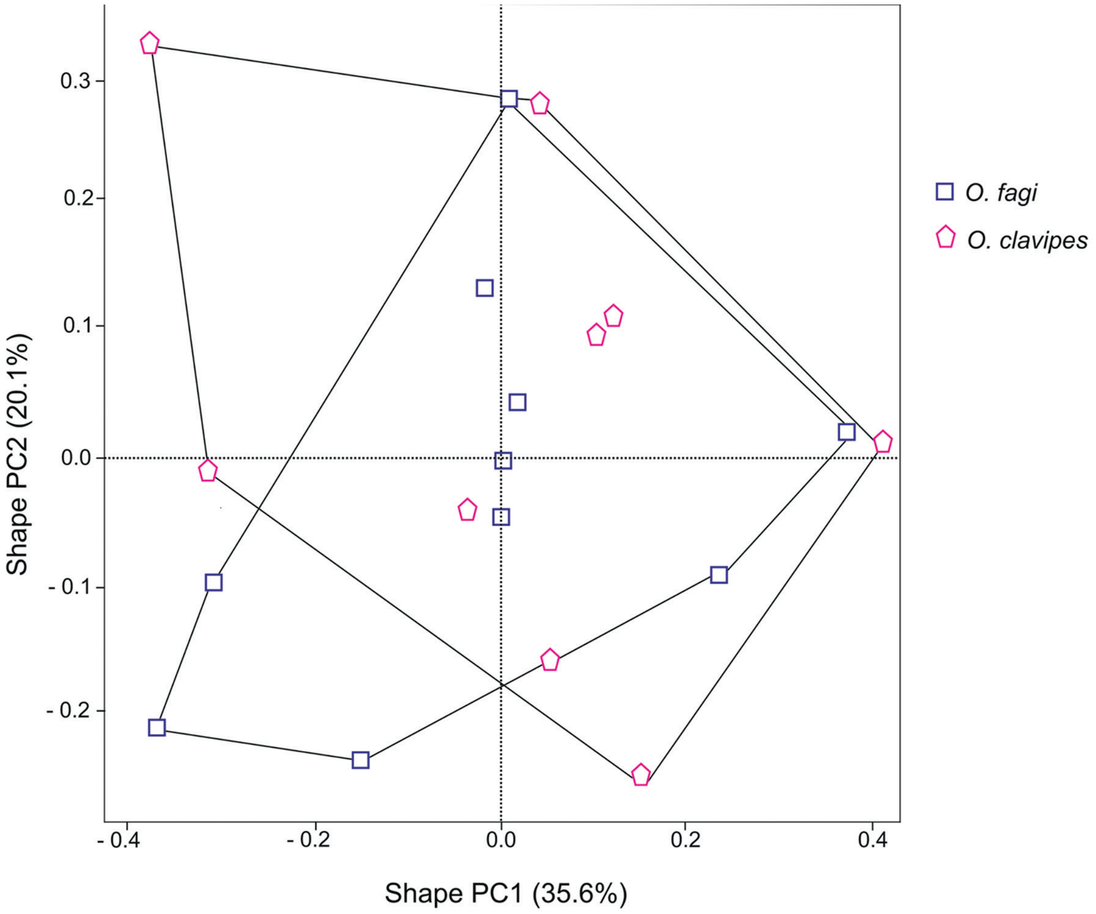


Fig. 7. Shape principal component analysis (PCA) for exploring females' variation within *Otiorhynchus fagi* (□) and *Otiorhynchus clavipes* (⌂) using all variables, the variance explained by each shape principal (PC) is given in parentheses. Statistical analysis showed no significant differences.

There are no reliable data for Great Britain, Ireland, the Netherlands, and North America. The data for Denmark have been revised by Casalini and Colonnelli (2019) and belong to *O. clavipes.* Records from Belgium are probably confined to the eastern parts, where altitudes of over 650 m a.s.l. are achieved (M. Delbol, Liège, pers. comm. 2020). Records from Luxembourg seem possible as heights above 500 m are achieved, too, but should be confirmed. All records from four Dutch provinces belonged to *O. clavipes* (Heijerman 2020). In Slovenia *O. fagi* is present but apparently not common (M. Kahlen, pers. comm. 2020). Records from Spain (Alonso-Zarazaga 2018) are all from the Basque region bordering to the west of the Pyrenees: two old and one recent record from 2019, identified by Bahillo de la Puebla as *O. tenebricosus* (MA Alonso-Zarazaga, pers. comm. 2020), may be correct, but the finding region is situated rather far and isolated from the next occurrences in France. Thus it remains doubtful if *O. clavipes* can be really excluded and if this occurrence is secondary (introduced) or represents an original site. Information on the introduction to North America, reported in the catalogue of Alonso-Zarazaga et al. (2017), is probably not correct. The basis of this record is unknown. Our research revealed only data about an introduction of *O. clavipes* to North America, according to Poole & Gentili (1996).

Biogeographical element. Western European: eastern part, Central and Eastern European, in mountainous regions, abundant along the chains of the Alps, including Maritime Alps, the Carpathians, Sudetes; further west until Massif Central; further north in the mid-mountain areas of Germany and Belgium, and further south along the Italian mountain chain of the Apennines; disjunct occurrences apart from this core area were reported for several regions. Introductions of this species are rare – opposite to *O. clavipes*. In the light of the Dutch *O. tenebricosus* records, just recently determined by Heijerman (2020) as *O. clavipes*, the distribution of both species in Belgium, which was communicated preliminairily by M. Delbol (pers. comm.), should be confirmed and shown more in detail.

Altitudinal distribution. It occurs between 400 m and 2500 m above sea level (a.s.l.), and it is most abundant and frequent between 800 m and 2100 m a.s.l.

Otiorhynchus* (*Otiorhynchus*) *clavipes (Bonsdorff, 1785)
(Figs 9–10, Table 5)

Synonyms were presented according to the order of data descriptions of species names, after Alonso-Zarazaga et al. (2017) and Casalini & Colonnelli (2019):

= *Curculio clavipes* Bonsdorff, 1785: 40
= *Curculio rufipes* Sturm, 1792: plate 17 not *Curculio rufipes* Linnaeus, 1758
= *Curculio maritimus* Donovan, 1811: 63 not *Curculio maritimus* Marsham, 1802
= *Othiorhynchus substriatus* Silbermann, 1833: no. 7
= *Otiorhynchus substriatus* Gyllenhal, 1834: 563, not *Othiorhynchus substriatus* Silbermann, 1833
= *Otiorhynchus lugdunensis* Boheman, 1842: 268
= *Otiorrhynchus elongatus* Stierlin, 1861: 65 not *Otiorhynchus elongatus* Hochhuth, 1847
= *Otiorhynchus francolinus* L.W. Schaufuss, 1867: 22, replacement name for *Otiorrhynchus elongatus* Stierlin, 1861: 65 not *Otiorhynchus elongatus* Hochhuth, 1847
= *Otiorhynchus longulus* Marseul, 1872: 250, replacement name for *Otiorrhynchus elongatus* Stierlin, 1861 not *Otiorhynchus elongatus* Hochhuth, 1847
= *Otiorhynchus longulus* Marseul, 1873: 143
= *Otiorrhynchus dilatipes* Guillebeau, 1885: 2
= *Otiorhynchus guillebeaui* Desbrochers des Loges, 1894: 89
= *Otiorrhynchus clavipes* ssp. *evertsi* Uytenboogaart, 1931: 292

Adult morphology. *Otiorhynchus clavipes* is generally morphologically very similar to *O. fagi*, but especially specimens up to now identified as *O. lugdunensis* are stronger and broader in habit. The body is 8–15 mm long, black and covered with hairs clear. The head is black, often cherry colored behind the eyes, with small but clearly visible, light colored scales between the eyes and on the entire upper surface of the rostrum. The granules on pronotum and elytra are fine. The elytra are about twice as long as wide, usually loosely covered with groups of small, silver-gray spots clearly visible in the usually faint grooves, sometimes bare even on the disc. The femur is widened in the middle part, cherry, without spurs. The tibia is black.

Male. The elytra are elongate. The last abdominal sternite (AS) is with about 6–15 strong ribs in the middle, on the sides with different numbers of very fine smaller ribs and grooves, and in front of the end with a well-developed cavity; at the hind-margin there is a dense fringe of erect yellow hairs. The aedeagus is almost parallel in the apical area, and the apex is angular, the inner sac is almost completely reduced. The tip of the spiculum ventrale is more or less deep concave (Germann 2013; Schütte et al. 2013).

Female. The elytra are oval. The last abdominal sternite usually bears punctures and hairs.

Larva. The head is dark yellow; all thoracic and abdominal segments are from yellowish to brownish; cuticle is almost smooth (Gosik et al. 2016).

Ecology. This species occurs in the forest zone of colline regions and in medium to high areas, but mainly in the low mountain ranges of Western and western Central Europe. In the northern central and eastern part of the continent (e.g., Northern Germany, Poland), it was introduced and inhabits mainly urban estates (cities, very rarely outside), such as gardens, parks, cemeteries, nurseries and similar areas. This species prefers several Oleaceae, and it has been found mainly on common lilac (*Syringa*), but also on privet (*Ligustrum*), young ashes (*Fraxinus*), *Euonymus*, *Thuja* and a few other bushes, which are also involved in passive transport processes and the introduction to other regions by trade. Adults are polyphagous and nocturnal. Weevils start to eat the leaf margin of shrubs at dusk and continue by night. Larvae are also polyphagous and develop mainly between the roots of these plants. They were bred with *Euonymus fortunei* (Turcz.) Hand.-Mazz., *Ligustrum vulgare* L. and *Syringa vulgaris* L. (Gosik et al. 2016).

The last (sixth) larval instar forms a pupal chamber with compressed walls made from soil under the ground (Gosik et al. 2016). Metamorphosis of the larvae occurs from end of February, and in the Paris region adults emerged from the pupa in March (Hoffmann 1950). According to Ibbotson & Edwards (1954), the life cycle of *O. clavipes* is completed within 12 or 18 months depending on the oviposition period. Adult weevils appear in two periods. One part pupates in autumn, overwinters in the pupal chamber and emerges in large numbers from April to May, the other part pupates from mid-May to July, and adults emerge from mid-June to end of August. For pupation larvae move deeper in the soil and pupate between 15 and 20 cm below soil level. According to Gosik et al. (2016) early emerging weevils lay eggs in spring and early summer, larvae develop during spring and summer, pupate and hatch in autumn, overwinter in the pupal cell and emerge again early in the season. And weevils laying eggs later in the season larvae overwinter and complete their development in the following season.

Food plants. Larvae and adults are polyphagous. Mainly feed on common lilac, *Syringa vulgaris*, and also *Ligustrum* sp., *Aucuba* sp., *Lonicera* sp., etc., especially in Isère (France) they were observed on vine (Hoffmann 1950). Beetles were collected from various shrubs, such as *Euonymus fortunei* (Turcz.) Hand.-Mazz., *E. japonicus* Thunb., *Fraxinus excelsior* L., *F. ornus* L., *Viburnum*

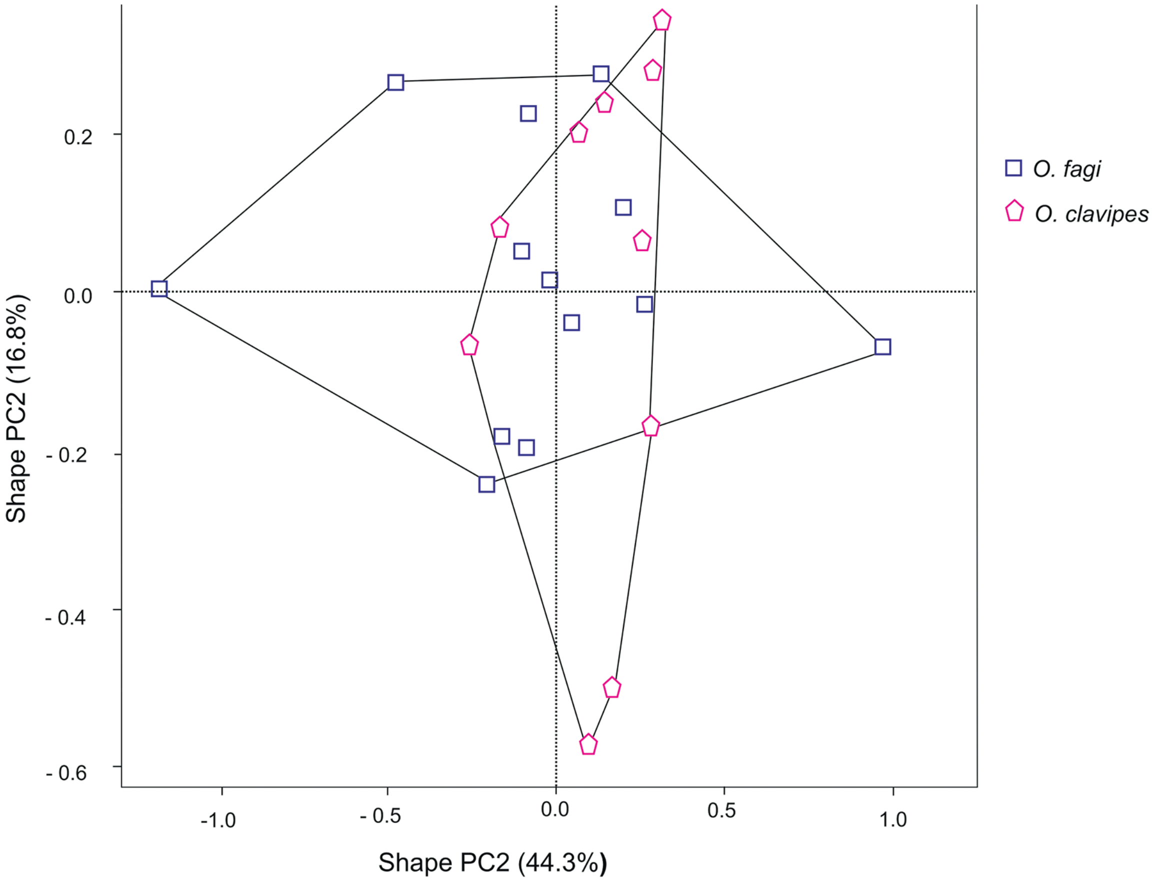


Fig. 8. Shape principal component analysis (PCA) for exploring males' variation within *Otiorhynchus fagi* (□) and *Otiorhynchus clavipes* (⌂) using all variables, the variance explained by each shape principal (PC) is given in parentheses. Statistical analysis showed no significant differences.

tinus L., *V. opulus* L., *Acer* sp., *Rhus coriaria* L., *Weigela* sp., *Vitis* sp., *Lonicera* sp. and *Thuja plicata* Donn ex D. Don (Hoffmann 1950, 1963; Gosik et al. 2016; Stüben et al. 2015). In the past *O. clavipes* was reported by Hoffmann (1950, 1963) to be harmful to *Syringa* sp. and *Ligustrum* sp. in nurseries, and present on *Aucuba* sp., *Viburnum tinus*, *V. opulus*, *Acer* sp., *Rhus coriaria*, *Weigela* sp. and *Vitis* sp. Ibbotson & Edwards (1954) in turn mention this species as noxious to English strawberry cultures in a rather small area around Cheddar, only. In Northern Germany it was regarded as species with no or nearly no economic importance in all parks and tree nurseries where it was found during 2008 to 2011 (Sprick & Stüben 2012).

Appearance of the adults. In the nightfall adults begin to devour the edges of buds and leaf plates and gnaw already in April and May on the leaf and flower buds of common lilac. In the course of the year large notches are eaten in the leaf margin of different bushes, causing characteristic incisions. The weevils already copulate in mid-April and start laying their eggs in the ground more or less during the entire season (Gosik et al.2016). They were found on the plants from April to mid-August (Hoffmann 1950), with maximum abundance in May and June.

Geographical distribution. The locus typicus of "*O. lugdunensis*" is Lyon (Hoffmann 1950). This species, now *O. clavipes*, shows a western to western central European distribution. Moreover it was introduced to areas around the North Sea and the Baltic Sea, to eastern Europe, and even to North America.

In France, it is widespread throughout the entire country, mainly in the plains and low-altitude hills but also in the higher mountain ranges (e.g.: Cevennes, Ardèche, la Garde, la Lozère, montagnes de Provence, Basses-Alpes, Alpes Maritimes, and also in many other regions (Hoffmann 1950; GBIF https://www.gbif.org); there are also observations from the area around Paris, where it can sometimes be harmful to nurseries (Hoffmann 1950; Zagatti et al. 2001), which may also depend on introduction.

In Germany it was introduced to many localities in the lowlands, especially into the northern sandy plains, e.g., Lower Saxony, Schleswig-Holstein, Brandenburg, Sachsen (Dieckmann 1980; Sprick & Stüben 2012; Schütte et al. 2013; Stüben et al. 2015).

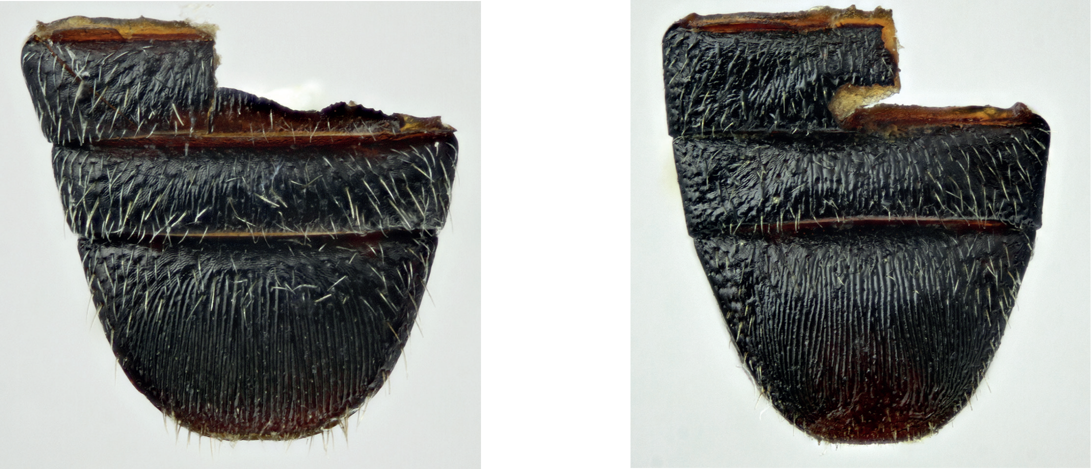

Otiorhynchus fagi

Otiorhynchus clavipes

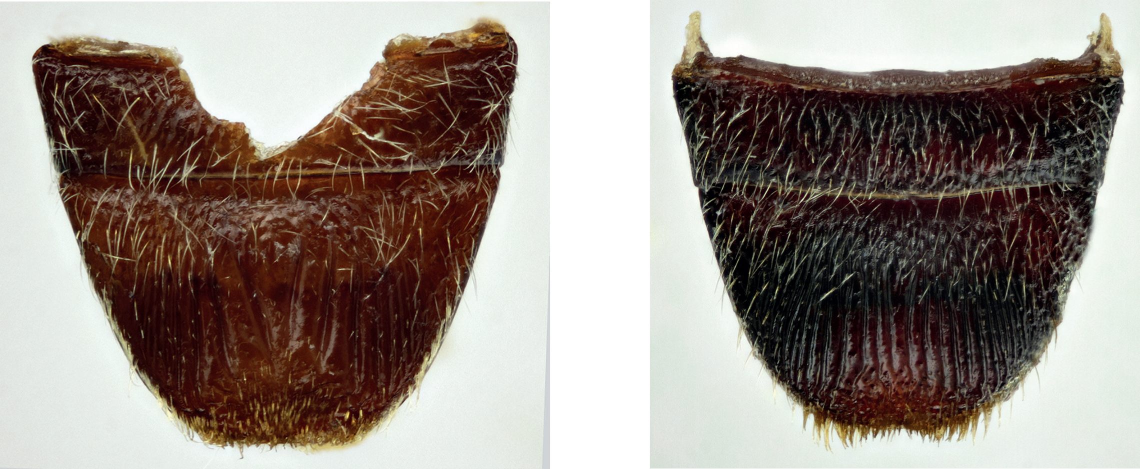

Otiorhynchus clavipes (U.K.)

Otiorhynchus coecus

Fig. 9. Habitus of males. *O. fagi* (Germany, National Park Harz, Sachsen-Anhalt: Brocken, experimental garden, 7.8.2019, 1100 m a.s.l., leg. & det. P. Sprick; bl = 9.9 mm, bw = 3.95 mm, hw = 1.675 mm), *O. clavipes* (Germany, Hannover-Linden, park south of Leine river, 25.5.2009, leg. & det. P. Sprick; bl = 11.2 mm, bw = 5.05 mm, hw = 1.9 mm), *O. clavipes* (U.K., England, Cornwall: Rescassa, 21.5.1989, leg. & det. M.G. Morris; bl = 9.55 mm, bw = 4.1 mm, hw = 1.7 mm), and *O. coecus* (Germany, National Park Harz, Sachsen-Anhalt: Brocken, experimental garden, 28.5.2019, 1100 m a.s.l., leg. & det. P. Sprick; bl = 8.2 mm, bw = 3.6 mm, hw = 1.5 mm) as an outgroup; bl: body length, bw: body width, hw: head width. All photos by F. Bahr. Scale bar = 5 mm.



Otiorhynchus coecus *Otiorhynchus fagi*



Otiorhynchus clavipes (U.K.) *Otiorhynchus clavipes* (France)

Otiorhynchus clavipes

Fig. 10. Abdominal sternites (AS) of males. *O. coecus* (Germany, National Park Harz, Sachsen-Anhalt: Brocken, experimental garden, 28.5.2019, 1100 m a.s.l.; AS: 1.65 × 0.9 mm (= 1.83:1), as an outgroup; *O. fagi* (Germany, National Park Harz, Sachsen-Anhalt: Brocken, experimental garden, 7.8.2019, 1100 m a.s.l., both leg. & det. P. Sprick; AS: 1.65 × 0.9 mm (= 1.83:1); *O. clavipes* (U.K., England, Cornwall: Rescassa, 21.5.1989, leg. & det. M.G. Morris, the brownish colour indicates a specimen with not fully-sclerotized exoskeleton; AS: 1.9 × 1.15 mm (= 1.65:1); *O. clavipes* (France, Savoie: Valloire, 19.8.1987, leg. H. Günther, det. P. Sprick; bl = 9.8 mm, bw = 4.45 mm, hw = 1.75 mm; AS: 1.9 × 1.1 mm (= 1.73:1); *O. clavipes* (Germany, Hannover-Linden, Park south of Leine river, 25.5.2009, leg. & det. P. Sprick; AS (2.3 × 1.3 mm = 1,77:1); bl: body length, bw: body width, hw: head width. All photos by F. Bahr.

In Poland, *O. clavipes* is known only from a few urban areas, like Wrocław and Olsztyn (Białooki 2005; Mazur 2016; Wanat & Mokrzycki 2018). Its occurrence in large towns or in areas with tree nurseries is very typical for introduced flightless weevil species.

Heijerman (2020) confirmed presence *O. clavipes* in the Netherlands where it was recorded in several provinces. He supposed that it is not introduced there.

The known current distribution of *O. clavipes*, mainly based on Alonso-Zarazaga et al. (2017), is revised here as follows: originally occurring in Belgium, France, Great Britain, Germany (western parts: Baden-Wuerttemberg, Hesse, North Rhine Westphalia; Köhler & Klausnitzer 1998), Ireland, Luxembourg, the Netherlands (southern colline part), and introduced to: Denmark, Estonia, Germany (northern parts), Latvia, the Netherlands (plain regions), Poland, where it is sometimes very rare and local (Mazur 2016), Sweden and Switzerland (Germann et al. 2017). The few records from Switzerland are based on introduction (C. Germann, pers. comm. 2020).

Similar as in *O. fagi*, the few extreme southwestern records of *O. clavipes* are from the Pyrenees and adjacent areas, two on the French and two on the Spanish side, usually old records, remain doubtful. And if correct, there is no information, whether these records from Spain and the French Pyrenees are based on original occurrence or on introduction.

Biogeographical element. Western European including the western parts of Central Europe; lower foothill (colline zone) to mountainous regions, introduced in the lowlands (usually < 100 m a.s.l.) in northern Central, southern North and Eastern Europe, rarely elsewhere, often on sandy ground; introduced to North America (Poole & Gentili 1996) (Table 5).

Otiorhynchus hungaricus Germar, 1824

Synonyms after Casalini & Colonnelli (2019):

= *Otiorhynchus hungaricus* Germar, 1823: 351:
= *Otiorrhynchus hungaricus* var. *hospitellensis* Hustache, 1923: 54

The third species of the *O. clavipes* group is *O. hungaricus*, which was not main study object of this paper, but it is included in the revision of the distribution, here.

According to Alonso-Zarazaga et al. (2017) the range of *O. hungaricus* was reported as follows: Bosnia-Hercegovina, Bulgaria, Croatia, Russia: Central European Territory, Germany, Hungary, Moldova, Romania, Serbia, Slovakia, and Ukraine. As there are no data for Germany, this often repeated record has to be deleted from the list until an introduction may have been proven.

The following records from isolated sites, which are given by different authors via https://www.gbif.org, have to be assessed in future, as to whether they are native or based on introduction: Estonia (Tallinn-Lasnamäe, limestone grassland, several specimens) by Roosileht (2015), Poland (Góry Stołowe, Sudetes Mts., > 800 m a.s.l.) by Wanat (2017), and Czech Republic (Třinec, around 300 m, surrounding area up to over 900 m a.s.l.) by Langeveld et al. (2020). The Estonian records are so far north of the core area (> 1000 km) that an introduction seems probable, even if the species inhabits limestone grassland in this northern exclave. The record from Poland is from a rather high area, untypical for this species. The presence in France is limited to the extreme Southeast (region of Digne and Alpes-Maritimes; Hoffmann 1950), but there is no information about habitats, indicating that its status remains unresolved, probably this is a historical introduction of an Eastern European species. In western Ukraine *O. hungaricus* inhabits mainly the low mountain Carpathians foothill zone, but the upper altitudinal limit is unknown. The Russian territory is situated also rather far from the core area of this species, and introduction cannot be excluded. The presence in Moldova confirms the preference of lower foothill sites, as the highest mountain of this country achieves only 428 m a.s.l. Apparently very little is known about host plants (polyphagous, preference for Rosaceae, according to Mazur 2002) and altitudinal limit. The determination of the right biogeographical character and especially in the northern sites the status as native or introduced species have to be regarded as provisional. Benedikt et al. (2010) listed this species only from one site in Slovakia (Matranské Pannonikum region) and characterized it as forest species of steppe regions. Thus, we regard it preliminarily as Southeastern European forest steppe species that prefers lower altitudes.

In summary it can be stated that there are well-developed characters of aedeagus and on abdominal sternites of males (Fig. 8) and very subtle differences, sometimes easy to overlook, between certain parts of the body of *O. fagi* and *O. clavipes*, such as the presence or absence of elytral spots (Table 5, Figs 7–8), and that there are significant genetic differences between *O. fagi* and *O. clavipes* (Tables 3–4, Figs 1–2) and separate geographical ranges in Central Europe between all three studied species.

DISCUSSION

The results of our study supports the legitimacy of the two separate species, *O. fagi* and *O. clavipes*, which had been also suggested by previous studies (Germann 2011, 2013; Gosik & Sprick 2013; Schütte et al. 2013; Stüben et al. 2015; Gosik et al. 2016; Casalini & Colonnelli 2019; Smreczyński 1966; Dieckmann 1980), in opposite to synonymizing both taxa proposed by Magnano (2001) and Fauna Europaea (Alonso-Zarazaga 2014). The data indicate that *O. fagi* is a mountain species (Massif Central, Alps, Carpathians, Sudetes) and several other more or less adjacent mountainous areas of Europe with an enlarged distribution rather far to the North and to the South in the central part of its range. *Otiorhynchus fagi* inhabits preferably coniferous, and also deciduous forests and related clearings with shrubs, young trees and tall herbs. On the other hand, *O. clavipes* is a Western to western Central European species inhabiting mainly areas with deciduous trees.

Weevils used in molecular analysis were collected in various regions of Germany, Poland, Slovakia and Slovenia and marked a priori as *Otiorhynchus clavipes* (C), *O. lugdunensis* (L) and *O. tenebricosus* (T) according to the usual nomenclature in the beginning of 2019 and to available identification keys (Table 1), previously to publication of Casalini and Colonnelli (2019). Our results (Figs 1–4, 9–10) confirm the synonymization of *O. clavipes* and *O. lugdunensis* and are consistent with the recently published taxonomic review of these authors (Casalini & Colonnelli 2019).

Considering the limitations inherent to an exceptionally morphological study of species delimitation, we selected an integrative approach combining genetic, molecular and morphological methods. The desirability of an integrative taxonomy has been acknowledged for more than a decade (Dayrat 2005; Valdecasas et al. 2008; Ober & Connolly 2015), even if it became apparent that observations of the chromosomes did not help in the separation of taxa, probably because it is a symplesiomorphic trait (Holecová et al. 2002; Lachowska et al. 2006). Measurements of several body parts did also not help very much in our study (Figs 7–8), although these methods were widely used in the past and very useful in discovering morphological diversity (e.g., Marvaldi et al. 2002; Przybycień & Wacławik 2015). Differences in some morphological features between both taxa were observed but they were not statistically significant. Reasons for the differences of most morphological features between *O. fagi* and *O. clavipes* being so small could be the phylogenetically low age and their close relation. Both species produced a rather high number of varieties indicating rather high speed of differentiation maybe due to Pleistocene or pre-Pleistocene spatial isolation in mountain areas and indicating a low rate of intermixing despite their size, which should allow a rather better spreading as in small flightless species. On the other hand this is in accordance with more recent spreading processes by the human-induced passive transport of *O. clavipes* as lowland and foothill species and the low introduction rate of *O. fagi* as mountain species. Moreover, both may be subject of ongoing speciation. It seems that genetic differences will accumulate in prospective generations, which may lead to even greater isolation between both taxa and greater morphological differences.

For many weevil species there are significant similarities in morphology, and they can be distinguished mainly based on their genetic traits, like the genus *Austromonticola* and new species from New Zealand (Brown 2017) or the genus *Sitophilus* with the two closely related species *S. oryzae* and *S. zeamais* (Correa et al. 2013), or some soil-inhabiting Cryptorhynchinae of the genus *Acallorneuma* and the tribe Torneumatini (Stüben et al. 2016). The method of DNA analysis used in our study also proved to be the most effective. The results of our molecular analysis with markers COI and EF-1α indicate that all German populations studied belong to *O. clavipes*, and populations from the Alps, the Carpathians and the Sudetes represent *O. fagi*. The populations of *O. fagi* from the Karawank Mts. (Slovenian Alps) are very different from *O. fagi* populations of other mountainous regions of Europe, at least based on the available COI data. We consider this a consequence of long time isolation. These divergences, especially in COI are rather uncommon in flightless species. Up to now COI or EF-1α could not be studied in specimens from France and England. Also habitus and abdominal sternite (AS) of *O. clavipes* from Germany, the United Kingdom and France show rather great morphological variation but differ even more clearly from the respective morphological parts of *O. fagi*.

Our results support the hypothesis that *Otiorhynchus fagi* and *O. clavipes* are two different species and that *O. clavipes* is mainly a Western European species. Its current presence in some cities in the eastern parts of Europe is an effect of introduction with common lilac, privet or other shrubs or young trees. Earlier suggestions of Dieckmann (1980) that diversification of the studied species began already in the Pliocene and was continued in the Pleistocene seem worth of further study. This kind of diversification is still going on, and we regard them as young, currently evolving species. Studies on evolution and phylogeography of these taxa in their entire range could more clearly explain process and time of this phenomenon.

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