Description of a new species of *Aphodius* Illiger from the Iberian Peninsula and comments regarding the biogeography and ecology of the subgenus *Liothorax* Motchulsky (Coleoptera: Aphodiidae)

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Abstract

A new species belonging to the genus *Aphodius* Illiger (subgenus *Liothorax* Motchulsky), *A. wilsonae* n.sp., is described from the Iberian Peninsula. The new species is diagnosed using both morphological characters and karyological and genetic evidence. In order to facilitate identification a revised key for the western Palaearctic *Liothorax* is provided. The ecology and biogeography of the group is discussed based on data accumulated by the authors from wild populations and collections.

Key words: Coleoptera, Scarabaeoidea, *Aphodius, Liothorax*, new species, chromosomes, DNA sequencing, Iberian Peninsula, western Palaearctic, Iran.

Introduction

The genus *Aphodius* Illiger currently contains more than 1,600 species (Dellacasa 1987) with over 190 species inhabiting Western Europe (Baraud 1992). This area has been well researched, hence few new species have been discovered in recent years. This paper reports the discovery of a previously unrecognised species belonging to the *A. niger* species complex.

The new species was originally discovered as part of a larger study on scarabaeoid chromosomes (Wilson 2002) from specimens collected in northern Spain (Balneario de Corconte, Burgos). The specimens were all collected under vegetable debris accumulated around the lake (strandline). The specimens were either in the soil-debris interface or slightly buried in the soil. Further collections from north central Spain, as well as museum collections, have allowed us to confirm the identity of this new species and to establish its distribution. In the present paper we provide a description of the new species based on morphological, karyological and genetic characters, and its comparison to its close relative *Aphodius niger* Illiger (this paper follows the submission to the ICZN by Krell et al. 2000).

Material and methods

The following collections were examined and type material has been sent to them. The authors would like to thank the curators for their help: The Natural History Museum, London, UK (BMNH) (Malcolm Kerley); Muséum d’Histoire Naturelle, Genève, Switzerland (MNHG) (Dr. Giulio Cuccodoro); Muséum National d’Histoire Naturelle, Paris, France (MNHP) (Mlle Nicole Berti); Naturhistorisches Museum Basel (incl. Georg Frey Collection, GFC), Switzerland (NHMB) (Dr. Eva Sprecher); Magyar Természettudományi Múzeum, Budapest, Hungary (HNHM) (Dr. Otto Merkl); Hope Entomological Collections, University Museum, Oxford, UK (HM) (James Hogan & Darren Mann); Robert Angus Collection (RA); Jason F. Maté Collection (JFM).
Chromosome preparations were obtained from testis and mid-gut cells, using the methods described by Shaarawi & Angus (1991) and Angus (1982). Preparations were photographed at 3000× and the images used to build the karyotypes. RCL (Relative Chromosome Length) was used to compare chromosome sizes in the karyotypes.

The sequences used in this paper are new and were obtained using the following protocol. Total DNA was extracted from Total DNA was extracted from a single, ethanol preserved specimen of each species analysed, by a phenol-chloroform extraction method (Vogler et al. 1993) or by DNAeasy ministep columns. The whole specimen (except for the abdomen) was digested overnight without grinding in a proteinase K solution, and the specimen retained as voucher. DNA amplification was carried out using the AmpliTaq DNA polymerase (Perkin Elmer) and the manufacturer’s magnesium solution (100mM Tris-HCL, pH8.3 at 25°C, 500mM KCl, 15mMgCl₂, and 0.01% w/v gelatin, autoclaved).

Both genes were amplified both ways using the primer pairs Pat-Jerry (850bp fragment of cytochrome oxidase I gene, primers Pat-5’ TCCAATGCACAAACTCTG GCCATATA and Jerry-5’CAACATTATTTTGTGTTT TGG) and a 425 bp fragment of cytochrome b (primers CB1 ff-5’TATGTACTACCATGAGA CAAAATATC and CB2 ff-5’ATTTACACCTCCTAATT TTAGGAAT).

Typical cycling conditions used were: one cycle of 2 min at 94°C followed by 35-40 cycles of 30 sec at 94°C, 30 seconds at 48–50°C and 40 seconds at 68–72°C, with a final elongation step of 10 min at 72°C. The ensuing products were cleaned using glassmilk (Geneclean II kit, Q-BIOgene, Ca, USA), vacuum dried and eluted in 10µl of distilled water.

Automated sequencing was performed using Applied BioSystems Ltd. Reagents (PRISM Ready Reaction Taq Cycle Sequencing, DyeDeoxy Terminator Reaction Kit) following the conditions recommended by the manufacturer. Ethanol-precipitated products were electrophoresed on an ABI377 sequencer. Sequences were edited using the Sequence Navigator software (Perkin Elmer). Comparisons were carried out in Paup b10 (Swofford 1998).

Chromosomes

The chromosomes of four of the species discussed are known, and all the karyotypes are distinctive. The Relative Chromosome lengths of the chromosomes (excluding dot-like y chromosomes) range from about 17–8 or 9.

*Aphodius* *plagiatus* (L.) (Fig. 1a–c). 2N = 18 + Xy. Chromosomes 1–4 and 6 are metacentric, while 5, 7–9 and the X chromosome are almost acrocentric, with the centromere near one end. The y chromosome is small, almost dot-like. C-banding shows heterochromatic blocks associated with the centromeres of all the chromosomes, with the y chromosome largely heterochromatic.
Fig. 2: First metaphase of meiosis from testis of *A. niger* from the New Forest (UK), C-banded.

*Aphodius niger* Illiger (Fig. 1d–h). 2N = 18 + XYp + B-chromosomes. This species differs from the other three in the large size of the Y chromosome. Chromosomes 1–5, and the X and Y chromosomes are metacentric, while the remaining pairs are more or less acrocentric, as are the B-chromosomes. C-banding shows that chromosome 1 and the X chromosome have long heterochromatic blocks which extend over more or less the whole of one arm. The heterochromatic blocks on chromosomes 3–5 are also long, but arranged symmetrically about the centromeres. The heterochromatic block of the Y chromosome is similar to that of chromosome 3. There may be up to three B-chromosomes, and these have distinct heterochromatic blocks at the centromere. Meiosis (Fig. 2) shows a cytoplasmic vesicle between the X and Y chromosomes, giving an XYp association.

*Aphodius wilsonae* sp.n. (Fig. 1i–n). 2N = 18 + Xy + B-chromosomes. The autosomes and the X chromosome are submetacentric, with one arm clearly longer than the other, this inequality of the arms varying between different chromosomes. The y chromosome is dot-like and the B-chromosome is a small submetacentric, similar in size to the X chromosome. Chromosomes 1–4, 6–8, the X chromosome and the B-chromosome have heterochromatic blocks occupying the whole of their longer arms.

*Aphodius paganettii* Petrovitz (*cylindricus* auct.), (referred to *Mendidaphodius* Reitter by Dellacasa & Dellacasa (2002) but retained in *Liothorax* here because the subgeneric classification in this case appears unclear, and it is included in *Liothorax* in the latest faunal work on the area (Baraud 1992)) (Fig. 1o–r). 2N = 18 + Xy. The y chromosome is dot-like. Chromosomes 2, 5 and 6 are metacentric, but the rest are submetacentric. C-banding shows the y
chromosome to be largely heterochromatic and all the other chromosomes to have heterochromatic blocks associated with the centromere. The long arm of the X chromosome is heterochromatic, and the short arms of chromosomes 1 and 2 also appear largely heterochromatic, possibly due to the presence of nucleolus organisers.

**Genes**

Four taxa were sequenced for the cytochrome oxidase gene (COI): *Aphodius niger*, *A. plagiatus*, *A. wilsonae* and *A. granarius* (L.) (the latter was used as an outgroup). Unfortunately it was not possible to sequence *A. paganettii* due to lack of material during sequencing. For the cytochrome oxidase gene (CB) it was not possible, despite repeated attempts, to obtain a sequence for *A. plagiatus*. Uncorrected pairwise divergences (p-distances) are shown in Table 1. As can be seen the pairwise divergences in the COI gene between the three species of *Liothorax* analysed are all very similar and comparable to the divergence between closely related species (MATÉ 2003) yet much higher than the intraspecific variability reported in other studies, which is less than 1% (ROSILIN 2001; MATÉ 2003; CABRERO-SAÑUDO & ZARDOYA 2004). Assuming a divergence rate of 2–2.3% per million years (DESSALLE et al. 1987), these divergences translate into a split between *A. wilsonae* and *A. niger* of 3–10 MYA.

The phylogenetic analysis of the data conclusively established that *A. niger* and *A. wilsonae* were sister taxa, with *A. plagiatus* placed consistently as sister to this grouping (exhaustive search, all characters included; tree length 213, CI =0.89, RI=0.88; branch support 13, bootstrap support 100%).

<table>
<thead>
<tr>
<th>% CB/COI pairwise distances</th>
<th>wilsonae</th>
<th>plagiatus</th>
<th>granarius</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>niger</em></td>
<td>4.94/10.72</td>
<td>na/10.46</td>
<td>16.94/14.62</td>
</tr>
<tr>
<td><em>wilsonae</em></td>
<td>-</td>
<td>na/11.58</td>
<td>16.71/18.87</td>
</tr>
<tr>
<td><em>plagiatus</em></td>
<td>-</td>
<td>-</td>
<td>na/13.84</td>
</tr>
</tbody>
</table>

Table 1: Percentage uncorrected p distance for CB (top) and COI (bottom) genes between all species sequenced.

The outgroup, *Aphodius* (*Calamosternus*) *granarius* was included to give a reference to the divergence between subgeneric groups. *Calamosternus* was determined to be closely related to *Liothorax* by MATÉ (2003) and CABRERO & ZARDOYA (2004).

*Aphodius* (*Liothorax*) *wilsonae* sp.n.

**Holotype:** ♂, length 5.0 mm, maximum breadth 2.2 mm. SPAIN, BU, Balneario de Corconte, 26.iv.2001 leg. R.B. Angus; Chromosome prep 6 3.v.2001; Holotype (small, round label with red rim); *Aphodius wilsonae* Maté & Angus, 2004 (BMNH).


Other material examined:
FRANCE: St Martin de Seignanx, Landes, Saint-Claire Deville (1 ex., MNHP); Sare, B. Pyr., IX.1948, leg. J. Aubry (1 ex., MNHP).
ITALY: Mte. Arazecca, Abruzzo, leg. Paganetti (1 ex., GFC); Aspromonte, P. Vaccarizzo, 20.IV.1971, leg. G. Dellacasa (1 ex., MG); Calabria, Santa Cristina, leg. Paganetti, Coll. R. Petrovitz (1 ♂, MG); Cippo Garibaldi, Aspromonte (3 exs., MHNHP); Calabria, leg. Pagani (1 ex., MHNHP); Sabde Salighes (1 ex., MHNHP).
BOSNIA: Giamos, Bosnia, ex Coll. R. Oberthür (2 exs., MNHP).
IRAN: Shiraz, L. Maharlou, 18.III.1965 (1 ♀, MNHP).

Small, cylindrical black beetle, Length 4.5–5.3 mm, breadth 1.9–2.2 mm. Head black, shiny, hemispherical with clypeus truncated to slightly emarginate, impressed medially; side angles rounded. Border of clypeus slightly raised. Genae produced beyond eyes, rounded. Puncturation double, obvious but fine, distance between the punctures 2–3 times diameter of the larger punctures. Large punctures more than twice the diameter of the small ones. Puncturation denser on the sides and genae, confluent at edge of clypeus in particular towards the sides, forming wrinkles. Only fine punctures on vertex. Frons elevated in the centre and divided from vertex by a smooth, unpunctured ridge; otherwise head unarmed.

Pronotum black and shiny; subquadrate to slightly elongate. Basal edge finely margined, margin sometimes disappearing towards the middle. Puncturation double, with large punctures over three times the diameter of the smaller ones. Large punctures denser on sides and towards front angles. Large punctures with bottom flattened.

Elytra black, shiny but shagreened, obvious under high magnification (> 30×). Interstices with very fine punctures in two parallel rows, visible under medium magnification (20×). Striae one fifth the width of the interstices, with deep punctures emarginating the edges, separated by between three and four times their diameter.

Legs black to fuscous, tibiae sometimes dark red. Front tibiae of male with spur more curved, reaching just beyond middle of second tarsal segment. Female with the spur less strongly curved, reaching apex of second tarsal segment. Metatarsal segments short. First segment subequal to or slightly longer than superior metatibial spur and shorter than next three tarsal segments together (Fig. 3.)

Underside completely black, with obvious yellow pubescence in abdomen. Metasternal plate, in males usually with obvious concavity in basal half, narrowing towards its distal end. In females, metasternal plate only impressed in the centre line. In both sexes punctation large and dense except towards the middle line. Epipharynx (Fig. 5b) similar to Aphodius niger (Fig. 5a) but chaetopedia with 6–8 spines on either side in Aphodius wilsonae, as opposed to 4–5 spines. Aedeagus: endophallus covered with small, short, spines (Fig. 4b), not large spines (Fig. 4a). Parameres slightly expanded dorsoventrally towards apex. Sensory areas in paramere apices poorly developed (Fig. 6a, b).

Etymology. The species is named after Dr. Christine Wilson, in the course of whose chromosomal investigations it was discovered.
Fig. 3: Metatarsi of a) *Aphodius wilsonae*; b) *A. niger*.

Fig. 4: Stereoscans of inflated aedeagi of a) *A. niger*; b) *A. wilsonae*.

Fig. 5: Epipharynx of a) *Aphodius niger* (Longslade, New Forest, UK); b) *A. wilsonae* (El Vellón, Spain); c) *A. isikdagensis* (Akrotiri, Cyprus).
Fig. 6: Aedeagus of *Aphodius wilsonae* sp.n. (holotype), a) dorsal and b) side view; c) *A. isikdagensis* (Limassol, Cyprus), dorsal view.

**Distribution**

By examining several collections it has been possible to obtain sufficient data to establish a rough distribution for the *A. niger*-species complex. From these data it appears that *A. wilsonae* and *A. niger* are allopatric (Fig. 7) although with further sampling areas were they co-occur may be found. The contact area seems to run along the Spanish-French border, Provence, northern Italy and the Balkans. The exceptions are two old *A. wilsonae* specimens, one from Thüringen (Germany) and one from Paris, both kept in the MNHP, and which may have been erroneously labeled. Regarding the distribution in the Italian Peninsula and the eastern Mediterranean, it is as yet not possible to clearly delimit the areas of both species due to insufficient material. As for the Iberian Peninsula, all specimens previously identified as *A. niger* have been determined to be *A. wilsonae*, with the exception of a small number of specimens from Vizcaya ("Vittoria, au Mt. Gorbea, R.Obr. & L.Bl., Juin 1879") in MNHG and MNHP.

Although the distribution of *Aphodius wilsonae* appears to be limited to western Europe, there is one specimen from Iran (Shiraz, 18.III.1965; *A. niger*: det. J. Baraud) which bears all the characteristics of *A. wilsonae*. Unfortunately it is a female and hence the authors cannot be certain of its identity. No other material from this area, either belonging to *A. niger* or *A.
wilsonae, has been found by the authors in collections. Hence it will be necessary to examine more collections before extending the range of A. wilsonae eastwards.

Finally, it is worth mentioning that material from North Africa identified as A. niger, has been determined by the authors to be A. isikdagensis. (Algeria Taguin, leg. De Vauloger, Nevinson Coll. 1918-14 BMNH 10 specimens; Tunis iv 83, Museum Paris 1935 Coll. M. Sedillot MHNP, 1 specimen). These represent new country records for Tunisia and Algeria. The only other Liothorax known from North Africa is A. plagiatus, and considering the confusion in identifying the species of this group, it will be necessary to re-examine previous A. plagiatus records using the discriminating features used in this article. Although we have not been able to examine A. plagiatus material from North Africa, one of the authors (RA) has examined A. plagiatus material from Gibraltar (Spain) in the BMNH collections and confirmed that they are indeed A. plagiatus. It is therefore possible that both species occur together in at least parts of North Africa.

![Geographical distribution of Aphodius niger (white circles), A. wilsonae (black circles) and A. isikdagensis (black squares) based on material examined in this study. Notice that the distribution of A. wilsonae is mostly Mediterranean with the exception of old records from Paris and Thüringen (MNHP).](image)

**Ecology of the subgenus Liothorax**

The ecology of the subgenus Liothorax has not been discussed a great deal in the literature, in particular the feeding preferences of the group. The breeding behaviour of the subgenus is unknown and most of the available information pertains to collecting records. In the case of A. niger, the species for which the most has been written, a preference for dung deposited by ponds.
or decaying vegetation has been noted (Jessop 1986). Adam (1994) and Lumaret (1990) considered A. niger (A. muscorum Adam) to be coprophagous, although both also reported a preference for humus rich, damp soils. On the other hand Jessop (1986) indicates that A. plagiatus is a saprophagous species, feeding on underground fungi in sandy areas, an opinion shared by Lumaret (1990), although he also reports some coprophily, as well as a preference for sandy, humus rich soils.

In the course of researching this paper, the authors have had the opportunity to collect four species in situ: A. wilsonae, A. niger, A. plagiatus and A. paganettii (now in Mendidaphodius). In all cases the adults were collected under vegetable strandline detritus and other dead plant material accumulated on the shores of water bodies, generally above the high water mark. The adults were found both in the soil interface as well as buried in the underlying soil, up to a few centimeters or so below the ground. Larvae were collected in the same situation as the adults for two species, A. niger and A. wilsonae. In the latter species one larva completed its development to adulthood when kept in the original strandline detritus, showing that they are able to utilize this as food. In addition collection records which include ecological data are also indicative of habitats consistent with saprophagy, such as salt marshes (e.g. A. niger: “E-Slovakia, Kopcianske luky nr. Malcice (salt meadows) 1-vi-1986 / David Král Lgt”; “Hortobagy N.P. Ujszentmargita/ Margital erdo egyeles/ 1975.x.20–22 leg. Migaly”; A. wilsonae: “Italy, Badde Salighe Sardegna”; A. isikdagensis: “CYPRUS: Akzotiri [ = Akrotiri] Bay 22.ii.1944 G. A. Mavromoustakis B.M. 1946-16”) and ponds (A. niger: all New Forest (Hampshire, UK) records). Thus it is likely that species of Liothorax are true saprophages, only rarely exploiting dung in the adult stage. This would explain their apparent rarity and difficulty of collection reported by authors (Lumaret 1990; Hortal & Cabrero 2002). The authors have found them to be fairly common locally when looked for in strandline detritus in late winter and early spring, but never in dung which was found close-by.

Key to the western Palaearctic Liothorax

The subgenus Liothorax currently includes seven species (Dellacasa 1987, Gusakov 2004) with another five recognised species listed in Mendidaphodius by Dellacasa & Dellacasa (2002). The following key has been designed to be used on the Western Palaearctic Liothorax only (Europe west of the border of the former USSR, plus Turkey and North Africa). Extension of the area covered to include all of European Russia would add A. rusakovi Gusakov to this list. This species is not included in the key as we have not seen any material. Gusakov (2004) describes it as resembling A. kraatzi (which he synonymises with A. haagi Becker, 1867, without explanation), but differing in the larger size (length 4.8–5.3 mm), stronger pronotal puncturation and modified protibial spur of the male. The characters used have been deemed to be the most reliable discriminators based on the samples to hand. Although Dellacasa et al. (2000a) make a complete separation between the two subgenera the distinction is ambiguous. Having screened the characters used by the authors as well as in Dellacasa & Dellacasa (2002), we have concluded that only the morphology of the clypeus gives a reasonably clear distinction.

1 Sides of emargination of clypeus angulate or dentate ......................................................... Mendidaphodius
- Sides of clypeal emargination rounded (Liothorax) .............................................................. 2

2 Elytral interstriae narrower (four times width of striae), nearly costiform in apex of elytra.
  Scutellum very wide, wider than second interstriae. Narrow beetles with elytral l/w ratio 1.44–
  1.46. ............................................................................................................................... kraatzi Harold
- Interstriae much wider, five times width of striae, normally convex, never costiform.
  Scutellum narrower than second interstriae. Elytral l/w ratio 1.30–1.38................................. 3
3 Metasternum with conspicuous white pubescence in anterior end and sides, in male with pronounced strongly pubescent median concavity. Elytra with obvious metallic sheen.......................... plagiatus (L.)
- Metasternum glabrous, or with inconspicuous yellowish pubescence. Male without prominent metasternal concavity. Elytra without metallic sheen except in A. wilsonae (but only when examined in alcohol) ................................................................. 4

4 First segment of metatarsus longer than upper tibial spur and subequal to next three segments. Male endophallus armed with large spines (Fig. 4a.). Chaetopedia with 4–6 spines (Fig. 5a)..................
- First segment of metatarsus shorter than upper tibial spur and equal to next to two segments. Male endophallus with only small spines (Fig. 4b). Chaetopedia with 6–8 spines (Fig. 5b, c)........... 5

5 Elytra obviously shagreened to the naked eye. Paramere tips flared (Fig. 6c) Clithra (anterior edge of epipharynx) obviously sinuate (Fig. 5c). Smaller, 4.0–4.2 mm..... isikdagensis BALTHASAR (= resslti BALTHASAR (DELLACASA et al. 2000b ), cypricola BALTHASAR)
- Elytra only slightly shagreened under high magnification. Paramere tips slightly rounded sideways (fig. 6a, b). Clithra straight (Fig. 5b). Larger, 4.5–5.3 mm........ wilsonae MATÉ & ANGUS

Acknowledgements

We thank Dr. Christine J. Wilson for permission to use her chromosome data on Aphodius plagiatus, A. niger and A. wilsonae. We also thank the British Council for a grant enabling the second author to visit Spain in 2001, and the Percy Sladen Memorial Fund for a grant enabling him to make a similar visit in 2002. We would also like to thank Polly S.P. Fong her kind assistance in the field, and Elizabeth Angus for help in collecting type material of A. wilsonae at Corconte.

References


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