Systematic Entomology

Systematic Entomology (2014), **39**, 531–547

Making the cryptic visible – resolving the species complex of *Aphodius fimetarius* (Linnaeus) and *Aphodius pedellus* (de Geer) (Coleoptera: Scarabaeidae) by three complementary methods

ANDREIA MIRALDO[1], FRANK-THORSTEN KRELL[2], MIRJAMI SMALÉN[3], ROBERT B. ANGUS[4] and TOMAS ROSLIN[3]

[1]Metapopulation Research Group, Department of Biosciences, University of Helsinki, Helsinki, Finland, [2]Department of Zoology, Denver Museum of Nature & Science, Denver, CO, U.S.A., [3]Spatial Foodweb Ecology Group, Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland and [4]Department of Life Sciences, The Natural History Museum, London, U.K.

Abstract. Species in cryptic complexes are, per definition, difficult to identify using morphological characters. One such complex was recently detected in the dung beetle *Aphodius fimetarius* (Linnaeus) sensu lato, an abundant dung beetle with a wide distribution. While the two component taxa, *Aphodius fimetarius* sensu stricto and *Aphodius pedellus* (De Geer) exhibit distinctly different karyotypes, the validity of subtle morphological characters proposed to distinguish between them has been debated. Given the variability and minor interspecific differences in external characters, the large-scale distribution of respective taxa has remained unknown, as have potential differences in ecology and habits. In this study, we ask how *A. fimetarius* and *A. pedellus* can best be distinguished, whether the use of different types of characters (karyotypes, DNA sequences and morphological traits) results in consistent species identification, where these species occur and whether they exhibit ecological differences. In total, we inspected a material of 4401 individuals from across the globe, of which 183 were examined for both mtDNA sequences and morphology, 154 for both morphology and karyotype, and 9 (including the recently proposed neotype of *Aphodius fimetarius*) for all three types of characters. As a marker gene, we sequenced a 590 bp region of the cytochrome *c* oxidase I gene for 183 individuals. Overall, DNA sequences offered a clear-cut distinction between taxa: sequences of *A. fimetarius* and *A. pedellus* differed by an average pairwise distance of 8.2%, whereas variation within species was only 0.9% for *A. fimetarius* and 0.5% for *A. pedellus*. Morphological and chromosomal characters offered species identifications consistent with that of molecular characters: karyotypes identified as *A. pedellus* consistently fell within one of the molecular clades, whereas karyotypes identified as *A. fimetarius* fell within the other clade. Likewise, the majority of individuals identified by morphological characters were assigned to the same species by sequence-based characters. Both taxa thus defined were found to be Holarctic in distribution, with major sympatry within Central and Southern Europe and mixed patterns of sympatry within the US. Northern areas of Europe, Asia and North America are dominated by *A. pedellus* alone. Within *A. pedellus*, patterns of sequence diversity

Correspondence: Tomas Roslin, Spatial Foodweb Ecology Group, Department of Agricultural Sciences, University of Helsinki, PO Box 27 (Latokartanonkaari 5), Helsinki FI-00014, Finland. E-mail: tomas.roslin@helsinki.fi

were indicative of a recent population expansion. In the western US, the phenology of a population of *A. fimetarius* was observed to significantly differ from that of a sympatric population of *A. pedellus*, thereby revealing an ecological difference between the two cryptic taxa. Overall, we conclude that all types of characters offered a consistent classification of the two species. Thus, the laborious karyotyping techniques used to originally establish the presence of two cryptic taxa can now be substituted by characters more easily applied to large ecological samples. Using this approach of integrative taxonomy, we were able to establish the global distribution and species-specific ecology of these ecologically important cryptic taxa.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid:zoobank.org:pub:4033473E-8BF7-40F4-852D-916E4F858593.

Introduction

Cryptic species are morphologically similar or identical taxa, which fulfil general criteria for achieving species status (cf. Bickford *et al.*, 2007; Detwiler *et al.*, 2010). Some cryptic species have diverged several million years ago without evolving any noticeable morphological or even ecological differences (Colborn *et al.*, 2001). In other cases, subtle morphological differences have been noticed once the existence of the species has been detected based on other characters (Glaw & Vences, 2002). Given that the traditional morphological species concept does not suffice to tell cryptic species apart, an estimated 2000 such taxa may await discovery – even among mammals (Baker & Bradley, 2006).

Importantly, the detection and description of cryptic diversity might change our perception of many aspects of species ecology, including species-specific distribution and/or abundance (Ashrafi *et al.*, 2010). The resolution of cryptic taxa may also affect our view of their conservational status: a species regarded as highly abundant and widely distributed may in fact prove a complex of several locally distributed cryptic taxa (Hebert *et al.*, 2004). As a specific concern, different cryptic taxa within a larger compound taxon may then show different population trends, with some taxa expanding and others retracting (cf. Kyle *et al.*, 2006 vs Murray & Waits, 2007). Such patterns could occur even when trend descriptors at the level of the compound species show no change.

Hidden cryptic richness is sometimes detected by chance, e.g. in association with chromosomal analysis (Wilson, 2001; Falahee & Angus, 2010). Yet, cryptic species are increasingly identified on the basis of DNA sequences (Hebert *et al.*, 2004; Hausmann *et al.*, 2009; Kaartinen *et al.*, 2010; Fontaneto *et al.*, 2011). In this context, DNA barcoding has been proposed as a fast and affordable tool (Hebert *et al.*, 2003a). The method is based on sequence variation within standardized marker genes (Hebert *et al.*, 2003a; Valentini *et al.*, 2009; Costa & Carvalho, 2010; Fontaneto *et al.*, 2011). In particular, the mitochondrial cytochrome *c* oxidase subunit 1 (COI) has proven a useful region in the resolution of animal taxa (e.g. Folmer *et al.*, 1994; Hebert *et al.*, 2003b; Valentini *et al.*, 2009). Robust universal primers have been developed for amplification of this region in diverse metazoan invertebrate taxa (e.g. Folmer *et al.*, 1994), and sequence variation has frequently proven larger between than within species (a phenomenon known as the barcode gap; e.g. Hebert *et al.*, 2003b; Ward *et al.*, 2005; Waugh, 2007; Bucklin *et al.*, 2011; but see e.g. Meyer & Paulay, 2005).

To offer reliable proof that cryptic taxa really correspond to 'good species', different characters proposed to delimit a set of cryptic species should ideally be cross-validated (Padial *et al.*, 2010). In some cases, reliable species identification may only be achieved through a combination of different types of characters (e.g. Lumley & Sperling, 2010). From a practical perspective, some methods of species identification call for major investment of time and effort in each individual, making them unsuitable for screening large ecological materials. As a comparison, chromosomal characters will call for a lengthy sample preparation (cf. Angus, 2006), whereas sequencing techniques are faster – and morphological characters clearly the fastest, at least when based on macroscopic and external characters. Here, one type of character may serve as a heuristic tool for discovering the cryptic species in the first place, but once a broader range of differences has been described among them, other characters may prove more easily applicable to specific situations (e.g. Barratt *et al.*, 1997; Thabah *et al.*, 2006; Funk *et al.*, 2012).

Aphodius fimetarius (Linnaeus, 1758), as understood by all authors before 2001, was a widespread and abundant dung beetle inhabiting the Holarctic, and small parts of the Oriental and Australian regions (Dellacasa & Dellacasa, 2003; Gordon & Skelley, 2007). At least in Northern Europe (Roslin & Heliövaara, 2009) and in the Nearctic region (Gordon & Skelley, 2007), it was identified as one of the most frequently collected dung beetles. Nonetheless, in 2001, it was surprisingly discovered that *Aphodius fimetarius* (Linnaeus) in fact comprises two cryptic species, *Aphodius fimetarius* sensu stricto and *Aphodius pedellus* (De Geer, 1774). While morphologically similar, these taxa display distinctly different karyotypes (Wilson, 2001; Wilson & Angus, 2004). No hybrid karyotypes have been found, even though the two taxa frequently occur in sympatry (Wilson, 2001). In addition, after the existence of two karyotypes

was established, minor morphological characters have been proposed to identify the corresponding taxa (Wilson, 2001; Whitehead, 2006; Rößner, 2012). Yet, the separation of the two taxa has not met approval by everyone, with e.g. Bordat (2002) and Dellacasa & Dellacasa (2003) calling for a re-synonymization of the two taxa.

In this study, we ask how *A. fimetarius* and *A. pedellus* can best be distinguished, whether the use of different types of characters (karyotypes, DNA sequences and morphological traits) results in consistent species identification, where these species occur now, whether they exhibit ecological differences, and whether their distribution has likely changed recently. More specifically, we first develop DNA barcodes to distinguish between the two species, and evaluate how species identifications based on these markers conform to those previously described as based on karyotypes and morphological characters. Second, we adopt the criteria for species identification thus validated to verify the distribution of the taxa at a both national and global scale. Third, we examine patterns of genetic differentiation in the two species for signs of a recent population expansion. Fourth, to screen for ecological differences among the two taxa, we compare their phenology in a strongly seasonal area where they co-occur. Finally, as the type specimen of *A. fimetarius* has recently been the subject of debate and a neotype proposed (Angus *et al.*, 2012; with a heated debate following: Ballerio, 2012; Barclay, 2012; Bellmann *et al.*, 2012; Bezdek & Král, 2012; Branco, 2012; Dellacasa & Dellacasa, 2012; Fery, 2012a,2012b; Forshage, 2012; Frolov, 2012; Krell & Angus, 2012; Maté, 2012; Roslin, 2012; Schmidt *et al.*, 2012; Solodovnikov, 2012; Fery, 2013), we provide the individual barcode of this very specimen, thereby anchoring the description of a cryptic species in an unequivocal molecular character.

Material and methods

To explore the characters distinguishing *A. fimetarius* sensu stricto from *A. pedellus*, and the global distribution of these taxa, we examined all material available at The Natural History Museum, London (BMNH); the Denver Museum of Nature & Science, Denver, Colorado (DMNS); the Field Museum of Natural History, Chicago (FMNH); The Hasbrouck Insect Collection at Arizona State University, Tempe, Arizona (HICASU); the private collections of C. J. Wilson (CJW) and Robert Angus, London (RBA); and some specimens from the C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins (CSU), the Erster Vorarlberger Coleopterologischer Verein, Bürs, Austria (EVCV); and the National Museum of Ireland in Dublin (NMID). In total, we inspected 4401 individuals of *Aphodius fimetarius* sensu lato from across the globe. Of these, all individuals were examined for morphology, 183 were examined for both mtDNA sequence and morphology, 154 for both morphology and karyotype, and 9 for all three types of characters. Detailed records of all individuals examined are given in Table S1 (Supporting Information).

Morphological identification

To establish whether morphological traits offered species identifications consistent with other characters, we identified all specimens using the diagnostic characters proposed by Wilson (2001), Whitehead (2006) and Rößner (2012; E. Rößner, personal communication). In no case was the outcome of the sequencing analysis known to the person (FTK) conducting the morphology-based identification. However, in the few cases ($n = 5$ out of 183 individuals examined for both mtDNA sequence and morphology; see below) where a mismatch between morphology- and sequence-based identification was observed, the specimen was returned to the examiner for an assessment of whether there was an actual conflict among the characters, or whether the first identification had included some errors in procedure or interpretation.

Once species identities had been cross-validated across all character types (see below), we returned to the original diagnostic characters, and revised them to remove all conflict between previously suggested morphological characters and verified species designations.

Karyotype information

Karyotype samples were prepared for 60 specimens of *A. pedellus* and 94 specimens of *A. fimetarius* following the protocol outlined in Angus (2006). In brief, we used a hypotonic inflation, air-drying technique on cells from mid-gut and testis. The material examined included individuals from Cyprus, Finland, France, Hungary, Ireland, Italy, Macedonia, Russia, Spain, The Netherlands, United Kingdom and United States of America (for exact details, see Table S1; for karyotype differences between *A. pedellus* and *A. fimetarius* see Fig. 1; Wilson & Angus, 2004).

DNA sequence information

A total of 183 beetles were sequenced for mtDNA COI gene. These individuals were sampled from across 64 sites in six countries (for exact details, see Fig. 2, Table 1 and Table S1). As we aimed to use molecular information both for species delimitation and for retracing the expansion history of the respective species, we invested specific effort in sampling populations across a range of latitudes within Northern Europe (Finland; 50 sites) and USA (six sites; Fig. 2). All unique sequences encountered in the material were registered in GenBank with accession numbers KJ740224-KJ740255.

DNA extraction, PCR amplification and sequencing

Preliminary trials using non-invasive techniques for DNA extraction offered surprisingly low yields and low PCR success (E. Vesterinen, personal communication). Thus, for the main part of the material, DNA was extracted from the head including whole prothorax and/or the first pair of legs. For this purpose, the tissue was frozen in liquid nitrogen and ground in a 1.5 mL Eppendorf tube. DNA was extracted using a NucleoSpin®

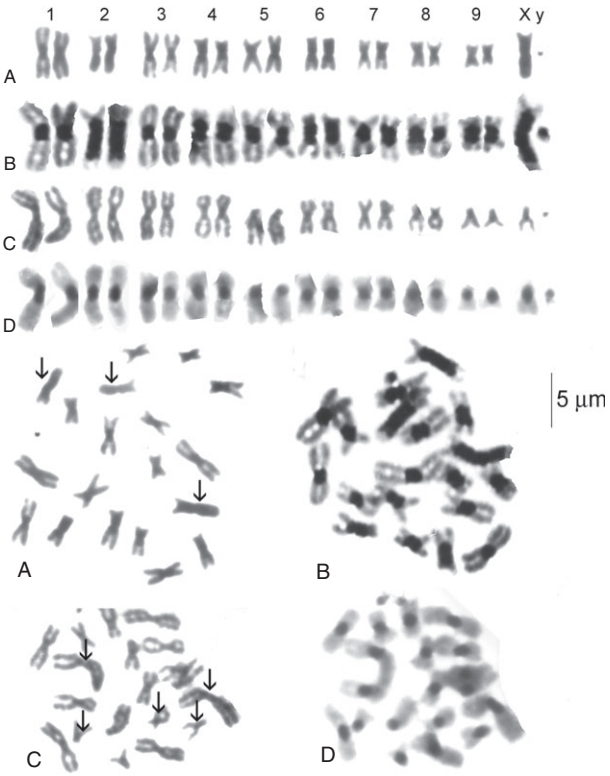

Fig. 1. Karyotypes of *A. fimetarius* and *A. pedellus*. Shown are the chromosomes of *A. fimetarius* Giemsa-stained (A) and C-banded (B) versus those of *A. pedellus* Giemsa-stained (C) and C-banded (D). The karyotype of *A. fimetarius* is characterized by wide C-bands across chromosome 2 and the X-chromosome, whereas that of *A. pedellus* is recognisable by the presence of two or three pairs of small acrocentric (centromere near one end) chromosomes, and by the large chromosomes clearly narrowed to a localized median centromere. For further information on karyotypic differences of *Aphodius fimetarius* and *A. pedellus*, see Wilson (2001) and Wilson & Angus (2004). Photographs taken by R. Angus.

Tissue extraction kit (Macherey-Nagel, Düren, Germany), following the protocol provided by the manufacturer. The quality and concentration of extracted DNA were measured with a NANODROP 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) prior to Polymerase Chain Amplification (PCR).

To generate DNA barcodes, the 710-bp Folmer-region of the cytocrome *c* oxidase subunit 1 gene (Folmer *et al.*, 1994) was amplified and sequenced with universal primers LCO1490 (5′-ggtcaacaaatcataaagatattgg-3′) and HCO2198 (5′-taaacttcagggtgaccaaaaaatca-3′ (Folmer *et al.*, 1994). PCR reactions were performed on genomic DNA using the standard PCR protocol recommended for KapaTaq DNA polymerase. Each reaction was performed on a final volume of 20 µL using 20–30 µg of DNA per reaction and PCR cycling conditions were as follows: denaturation at 95°C for 4 min followed by 40 cycles of 95°C for 30 s, 49°C for 30 s and 72°C for 1 min plus a final extension step of 72°C for 3 min. A negative control containing water (Milli-Q®) instead of template was used to control for contamination.

All PCR products were visualized by agarose gel electrophoresis (2%) and positive reactions were purified with an ExoSAP-IT® - purification kit (USB® Products, Affymetrix®, Inc., Santa Clara, CA, USA) prior to sequencing.

Purified PCR products were then sequenced in both directions using BigDye terminator v3.1™ (Applied Biosystems) chemistry on a Megabace 1000 automated sequencer (GE Healthcare). All sequences were manually edited and aligned with Geneious Pro (Drummond *et al.*, 2011). Given poor quality reads at the beginning and end of the majority of sequences, the final sequences were trimmed to 590 bp prior to analyses.

Sequencing of the type specimen

The neotype proposed for *A. fimetarius* is kept in the general Coleoptera Collection of The Natural History Museum, London, and has the reference number BMNH{E}UIN990028. This individual is henceforth referred to with its voucher code used in laboratory work, 'NEO' (cf. Table 1). To derive the exact DNA sequence of this individual, the back leg of the pinned beetle was carefully removed. DNA extraction was then performed at the University of Helsinki, using the QIAamp DNA Micro Kit® (QIAGEN, Valencia, CA, USA), which incorporates a protocol designed to extract DNA from small amounts of tissue. Once received, the sample was immersed in 200 µL of ATL buffer (provided with the extraction kit), and allowed to rehydrate overnight. Next morning, 20 µL of proteinase K was added, as followed by an extended digestion time of 18 h. After the digestion step, we followed the specific protocol provided by the manufacturer.

Analyses of genetic diversity

Intraspecific gene genealogies were inferred based on the maximum parsimony (Templeton *et al.*, 1992) as implemented in the program TCS 1.21 (Clement *et al.*, 2000) using a connection limit of 95%. The final network layout was created with the program HAPSTAR (Teacher & Griffiths, 2011). Estimates of sequence divergence were derived with the program Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). To obtain results directly comparable with Hebert *et al.* (2003b), we used the K2P distance model.

Patterns of sequence diversity and divergence within *A. fimetarius* and *A. pedellus*, respectively, were also used as proxies for recent population history. Rapid range expansion is expected to leave an imprint in terms of reduced molecular diversity (Excoffier *et al.*, 2009). An example from dung beetles is given by Hanski *et al.* (2008), who describes drastically reduced genetic diversity in beetle species switching to a new resource allowing rapid population increase. Naturally, an expansion following anthropogenic introduction will leave much the same imprint as any climate-driven expansion (Dlugosch & Parker, 2008), and thus, our analyses should be interpreted as targeting the effect rather than the cause of inferred population change. For this purpose, we compared absolute and relative levels of

Fig. 2. (A–C) Distributions of *Aphodius fimetarius* and *A. pedellus.* While the pooled distribution of this species complex covers major parts of the globe, the map focuses on areas where the identity of the species has been verified by the authors (see Table S1 for the material examined), and the published studies of Whitehead (2006) and Rößner (2012). For shaded areas, records have been attributed to the former compound species *A. fimetarius*, whereas for cross-hatched areas, records have been attributed to either *Aphodius fimetarius* sensu stricto or *A. pedellus* (for which, see figure legend). Red and blue dots represent records for which precise GPS coordinates could be obtained.

molecular diversity among the two species for patterns indicative of recent expansions.

To search for added signs of population expansion, we also examined the 'mismatch distribution', i.e. the frequency distribution of the observed number of differences between haplotype pairs in a population. This distribution is commonly ragged and multimodal in samples drawn from populations at demographic equilibrium. However, in populations which have passed through a recent demographic expansion, haplotype networks are typically starlike and centered on single common haplotypes, and the resultant mismatch distribution smooth and unimodal (Rogers & Harpending, 1992; Rogers, 1995; Wakeley & Hey, 1997; Ramírez-Soriano *et al.*, 2008, but see Marjoram & Donnelly, 1994). This relationship was used to assess whether current haplotypic variation in *A. fimetarus* and/or *A. pedellus* is consistent with demographic equilibrium (constant long term N_e), or whether it is better explained by the 'sudden expansion model' of Rogers (1995). The distributions of pairwise differences expected under the expansion model were calculated in Arlequin version 3.5.1.2 (Excoffier & Lischer, 2010). The agreement between observed and expected distributions was then evaluated using simple χ^2 statistics. Finally, to detect departures from a constant population size under the neutral model, we used Fu's F_s test statistics (Fu, 1997).

Phenology of sympatric populations

To determine whether *A. pedellus* and *A. fimetarius* differ in their ecology, we focused on phenology as an easily-measurable

character. Ten 1- to 2-day old, unmanipulated pats of bison dung were collected once a month during the snow-free time May–September from 2008 to 2012 at the Bijou Creek property of the Plains Conservation Center in Elbert County, Colorado, U.S.A. (39°32′08″–33′53″N, 104°15′16″–17′24″W; 1672–1717 m asl; short-grass prairie). In 2010 and 2012, a similar sampling protocol involving six cow dung pats per month was implemented at the Keen Ranch, Byers, Arapahoe County, Colorado, U.S.A. (39°35′39″–36′03″N, 104°16′03″–17′11″W; 1643–1721 m asl; short grass prairie rangeland). At both sites, beetles were quantitatively extracted by the floating method (Krell, 2007) and are deposited in the Denver Museum of Nature & Science.

To test for differences in phenology among species, and to examine the consistency of phenological patterns across sites, we modelled the counts observed as a function of Species (*A. pedellus* versus *A. fimetarius*), Site (Bijou Creek versus Keen Ranch), Month (a categorical variable with a separate value for each month from May to September) and the two- and three-way interactions among these variables. As we were dealing with counts, we assumed Poisson-distributed errors and a log link. The model was fitted in SAS System 9.2 for Windows, proc GLIMMIX (SAS Institute Inc., Cary, NC, USA).

Results

General patterns of DNA sequence variability

Thirty-two unique haplotypes were observed among the 183 sequences analyzed. All haplotypes represented uninterrupted open reading frames, with no gaps or premature stop codons. This pattern suggests that the sequences represent functional copies of the mitochondrial COI gene. Of a total of 590 sites sequenced, 53 were variable, of which 45 were parsimony-informative. Pairwise genetic distances (uncorrected p-values) between haplotypes ranged from 0.17 to 9.03%.

Genealogical relationships among COI haplotypes inferred by the parsimony network revealed two highly divergent groups of haplotypes (haplotypes H1–H15 and H16–H32) separated by 39 mutational steps with an average pairwise uncorrected genetic distance between groups of 8.18% (Figs 3, 4 and Table 2). Sequence variation was distinctly smaller within than between species, revealing the existence of a barcoding gap between *A. fimetarius* and *A. pedellus* (Fig. 3).

Do different types of characters offer consistent species identifications?

All types of characters offered mutually consistent species identifications. For the subset of eight individuals examined by both karyotyping and DNA-sequencing, the two major haplotype clades matched the two types of karytopes: karyotypes identified as *A. pedellus* consistently fell within one of the clades, whereas karyotypes identified as *A. fimetarius* fell within the other clade (Fig. 4). Likewise, the majority of 183 individuals identified by morphological characters were assigned to the same species by sequence-based characters. Discrepancies between morphological characters and sequence-based characters were detected in five specimens (DMNS ZE.15838, ZE.15839, ZE.31722, ZE.31738, und ZE.35091 in Table S1), which were morphologically identified as *A. fimetarius* but show a haplotype characteristic of *A. pedellus* (H1, H9 and H12). These specimens were found to be characterized by unusually smooth subapical areas of the elytra or covered by formerly unrecognized grease when rechecked by the original identifier. The neotype proposed for *A. fimetarius* (NEO in Table 1) was found to represent haplotype H20, and thus unequivocally fell within the clade of haplotypes characterizing *A. fimetarius* (Fig. 4). The full sequence of this individual was deposited in GenBank with accession number KJ740243.

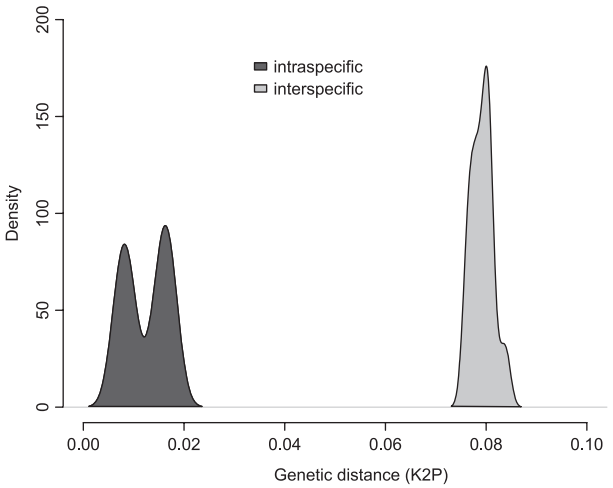

Fig. 3. Density distribution of intraspecific and interspecific (congeneric) genetic divergences in *Aphodius fimetarius/pedellus*. Divergences were calculated using the Kimura two parameter (K2P) model.

What morphological characters will distinguish between species?

Our iterative process of first identifying specimens based on proposed diagnostic morphological characters, then verifying species assignments by other characters, and finally revising the original diagnostic characters to remove any ambiguities revealed a set of morphological characters as distinguishing between species. These characters can be found in the frontal lobes of the head, in the microsculpture of the subapical area of the elytra, and in the shape of elytral intervals (Table 3; Fig. 5). Differences in elytral colour, aedeagal characters, and pronotal punctation as suggested by Wilson (2001), Whitehead (2006), and Rößner (2012) seem to fail when more material from the whole range is studied. The elytra of *A. fimetarius* are on average lighter than those of *A. pedellus*, but we found light and dark specimens in both species.

Table 1. Material used in molecular analyses. For each locality sampled, we show the number of samples collected (n), the COI haplotypes found at the respective locality, and the DMNS catalogue numbers or voucher id (for individuals not deposited at DMNS).

Locality		Country	Northing (WGS84)	Easting (WGS84)	n	Chomosome id	COI haplotype	DMNS catalogue numbers or voucher id
1	Asikkala	Finland	61.25164	25.40796	5	n.a.	H1, H14	ZE.15791, ZE.15792, ZE.15809, ZE.15824, ZE.15825
2	Elimäki	Finland	60.69349	26.39147	1	n.a.	H1	ZE.15872
3	Hämeenlinna	Finland	61.23703	24.56310	2	n.a.	H12, H14	ZE.15800, ZE.15853
4	Ilomantsi	Finland	62.93636	30.61837	1	n.a.	H1	ZE.31766
5	Juupajoki	Finland	61.78899	24.53431	2	n.a.	H1	ZE.15805, ZE.15858
6	Kannonkoski	Finland	62.95930	25.23817	1	n.a.	H1	ZE.15873
7	Kannus	Finland	63.88732	23.90091	2	n.a.	H1, H3	ZE.15812, ZE.15813
8	Karjalohja	Finland	60.19230	23.67007	3	n.a.	H1	ZE.15797, ZE.15820, ZE.15852
9	Kemijärvi	Finland	66.38773	27.28868	1	n.a.	H1	ZE.15869
10	Kihniö	Finland	62.25381	23.18997	2	n.a.	H1	ZE.15826, ZE.15874
11	Kitee	Finland	62.01503	30.04126	2	n.a.	H1, H8	ZE.15868, ZE.31767
12	Kittilä	Finland	67.67039	25.20654	1	n.a.	H1	ZE.31768
13	Kolari	Finland	67.10479	24.70203	2	n.a.	H1	ZE.15756, ZE.15821
14	Korppoo, Wattkast	Finland	60.18553	21.64006	5	*A. pedellus*	H1, H14	ZE.15817, ZE.15819, **ZE.31764, ZE.31765, ZE.35093**
15	Kortesjärvi	Finland	63.24638	23.05059	2	n.a.	H1	ZE.15870, ZE.31769
16	Kosken kartano	Finland	60.18150	23.28915	7	n.a.	H1, H7, H9, H14	ZE.15744, ZE.15745, ZE.15806, ZE.15807, ZE.15823, ZE.15831, ZE.15861
17	Kouvola	Finland	60.75983	26.95202	4	n.a.	H1, H2, H14	ZE.15802, ZE.15803, ZE.15804, ZE.15851
18	Lieksa	Finland	63.56150	29.57354	1	n.a.	H4	ZE.15747
19	Lohja	Finland	60.31400	23.88480	1	n.a.	H1	ZE.15746
20	Loppi	Finland	60.65983	24.47718	4	n.a.	H1	ZE.15789, ZE.15810, ZE.15811, ZE.15857
21	Merijärvi	Finland	64.35291	24.54459	1	n.a.	H1	ZE.15860
22	Miehikkälä	Finland	60.69875	27.45398	1	n.a.	H12	ZE.15798
23	Nilsiä	Finland	63.37371	28.23578	2	n.a.	H1	ZE.15844, ZE.35095 (Fig. 5B)
24	Nurmes	Finland	63.66044	29.09089	1	n.a.	H1	ZE.15822
25	Oripää	Finland	60.92117	22.59506	4	n.a.	H1, H14	ZE.15808, ZE.15827, ZE.15845, ZE.15862
26	Oulu	Finland	65.01885	25.42440	2	n.a.	H1	ZE.15863, ZE.31781
27	Paltamo	Finland	64.41658	27.67485	4	n.a.	H1	ZE.15748, ZE.15866, ZE.15867, ZE.31782
28	Parkano	Finland	61.92872	22.98456	5	n.a.	H1, H12, H14	ZE.15858, ZE.15864, ZE.15865, ZE.31770, ZE.31771
29	Pornainen	Finland	60.50864	25.29191	2	n.a.	H1, H12	ZE.15855, ZE.15856
30	Rääkkylä	Finland	62.23862	29.60020	1	n.a.	H13	ZE.15755
31	Ristiina	Finland	61.55188	27.46178	3	n.a.	H1, H14	ZE.15801, ZE.15846, ZE.15847
32	Rovaniemi	Finland	66.70210	25.46557	3	n.a.	H1	ZE.31772, ZE.31773, ZE.31774
33	Salo	Finland	60.56377	23.11028	3	n.a.	H1, H14, H15	ZE.15787, ZE.15790, ZE.15848
34	Siilinjärvi	Finland	63.01815	27.43479	2	n.a.	H1, H12	ZE.15753, ZE.15754
35	Simo	Finland	65.73600	24.95584	1	n.a.	H1	ZE.15871
36	Simo	Finland	65.74030	25.22185	2	n.a.	H1, H10	ZE.15749, ZE.15788
37	Somero	Finland	60.57869	23.36335	3	n.a.	H1, H12	ZE.15743, ZE.15799, ZE.15830

Table 1. Continued.

Locality		Country	Northing (WGS84)	Easting (WGS84)	n	Chomosome id	COI haplotype	DMNS catalogue numbers or voucher id
38	Sotkamo	Finland	64.01611	28.81712	1	n.a.	H1	ZE.15757
39	Suomussalmi	Finland	64.91070	28.80230	1	n.a.	H4	ZE.31775
40	Suomussalmi	Finland	65.36085	29.30466	2	n.a.	H1	ZE.15752, ZE.15816
41	Suonenjoki	Finland	62.51702	27.30753	1	n.a.	H1	ZE.31776
42	Tervola	Finland	66.10167	24.82903	1	n.a.	H1	ZE.15751
43	Tornio	Finland	65.97203	24.27604	1	n.a.	H1	ZE.15750
44	Ulvila	Finland	61.47912	22.15022	6	n.a.	H1, H3	ZE.15794, ZE.15795, ZE.15796, ZE.15843, ZE.15849, ZE.15850
45	Uusikaupunki	Finland	60.63559	21.53809	2	n.a.	H1	ZE.31777, ZE.31778
46	Valtimo	Finland	63.79214	28.64906	1	n.a.	H1	ZE.31779
47	Viikki	Finland	60.22808	25.01822	3	n.a.	H1	ZE.15793, ZE.15828, ZE.15829
48	Viitasaari	Finland	63.18845	25.95017	2	n.a.	H14	ZE.15814, ZE.35096 (Fig. 5H)
49	Ylitornio	Finland	66.31816	24.43977	1	n.a.	H1	ZE.31780
50	Ylitornio	Finland	66.30548	23.67065	2	n.a.	H1, H10	ZE.15815, ZE.15818
51	Elbert/Arapahoe Counties, Colorado	USA	39.55428	−104.26986	42	n.a.	H1, H5, H17, H18, H22, H27, H28, H31, H32	ZE.31715, ZE.31716, ZE.31717, ZE.31718, ZE.31719, ZE.31720, ZE.31721, ZE.31722, ZE.31723, ZE.31724, ZE.31725, ZE.31726, ZE.31727, ZE.31728, ZE.31729, ZE.31730, ZE.31731, ZE.31732, ZE.31733, ZE.31734, ZE.31735, ZE.31736, ZE.31738, ZE.31739, ZE.31740, ZE.31741, ZE.31742, ZE.31744, ZE.31745, ZE.31746, ZE.31747, ZE.31748, ZE.31749, ZE.31750, ZE.31751, ZE.31752, ZE.31753, ZE.31754, ZE.31755, ZE.31756, ZE.31757, ZE.31758
52	Custer County, Colorado	USA	38.21167	−105.44361	3	n.a.	H1, H31	ZE.31761 (Fig. 5A), ZE.31762, ZE.31783
53	Jefferson Co., Colorado	USA	39.75481	−105.10698	1	n.a.	H17	ZE.31760
54	Tehama Co., California	USA	40.34766	−121.60990	1	n.a.	H18	ZE.15842
55	Pocahontas Co., West Virginia	USA	38.16401	−79.97450	1	n.a.	H1	ZE.35091 (Fig. 5G)
56	Marin County, California	USA	37.99710	−122.52940	2	n.a.	H23	ZE.15836, ZE.31784
57	Box Hill, Surrey	UK	51.23845	−0.31750	6	n.a.	H1, H16, H19, H21, H29	BH1, BH2, BH3, BH4, BH5, BH6
58	Camber, East Sussex	UK	50.93845	0.78167	2	*A. fimetarius*	H20, H30	**ZE.15834, ZE.15841**
59	Great Mongeham, Kent	UK	51.21667	1.36667	2	*A. fimetarius*	H20, H26	**MUS, NEO**
60	Lac de Pontet, 1900 m	France	45.04856	6.33694	1	n.a.	H1	Pontet
61	Corsica, 1700 m	France	42.21298	9.01667	1	*A. pedellus*	H6	**ZE.15859**
62	Spain	Spain	40.40000	−3.68300	4	n.a.	H1, H13, H24, H25,	ZE.15832, ZE.15833, ZE.15835, ZE.15837
63	Madrid, Lozoya	Spain	40.95000	−3.78300	3	n.a.	H9, H12, H24	ZE.15838, ZE.15839, ZE.15840
64	Phakding, 2460 m	Nepal	27.94200	86.92528	1	n.a.	H11	ZE.35092

Bold font identifies samples examined for both molecular and chromosomal characters (with the species detected reported under 'chromosome id'), whereas for underlined samples, species were assigned to different species based on morphological versus sequence-based characters in double-blind tests (see main text for details). Locality numbers correspond to those used in Fig. 6, and COI haplotype codes to those used in Fig. 4. NEO, proposed neotype of A. *fimetarius*.

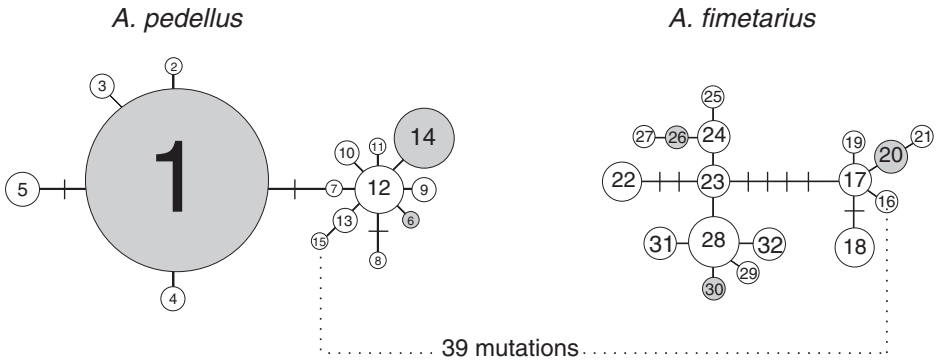

Fig. 4. Minimum spanning network of 32 haplotypes found among 183 COI sequences examined. Each circle represents an individual haplotype, its area proportional to its relative frequency within the total sample (the highest frequency being 112 and the lowest 1; note that scales differ between species). Each perpendicular line between two haplotypes represents one mutational event, short perpendicular lines along branches thus representing a 'missing' haplotype not detected in the sample. Haplotypes found in karyotyped individuals of *Aphodius fimetarius* and *A. pedellus* are indicated by grey shading. The proposed neotype of *A. fimetarius* has haplotype 20.

Where do the respective species occur?

Given that all three types of characters were found to offer reliable species identification, we used our combined material (i.e. all individuals as identified by one or multiple criteria) to evaluate the global distribution of the species. In total, the overall material of 4401 individuals of *Aphodius fimetarius* sensu lato yielded 3473 records of *A. pedellus* and 928 records of *A. fimetarius*. A first range map for *A. fimetarius* and for *A. pedellus* is offered as Fig. 2. Both taxa seem to be distributed all over the Holarctic and adjacent areas, with major sympatry within Central and Southern Europe and currently mixed patterns of sympatry within the US. Northern areas of Europe, Asia and North America are dominated by *A. pedellus* alone.

What do patterns of genetic differentiation reveal in terms of recent population change?

Within the respective species, somewhat different patterns of genetic divergence emerged: Overall, *A. fimetarius*, was characterised by a higher level of molecular diversity than was *A. pedellus* (Table 2). At the level of haplotype variety, we observed two more haplotypes in *A. fimetarius* than in *A. pedellus* (Table 2). As we actually sequenced much fewer individuals of *A. fimetarius* (n = 31) than of *A. pedellus* (n = 152), the slight discrepancy observed among samples seems indicative of a real difference in diversity among species, rather than of mere sampling bias. At the level of sequence divergence, the mean pairwise genetic distance among individuals was 5.19 substitutions (corresponding to 0.88% sequence divergence) in *A. fimetarius*, as compared to 2.92 substitutions (equalling 0.49% sequence divergence) in *A. pedellus*. The two species also differed in terms of haplotype frequencies, with the most common haplotype of *A. pedellus* accounting for a full 81% of conspecific individuals examined, compared to only 16% for the most common haplotype within *A. fimetarius* (Fig. 4).

Table 2. Molecular variation in 590 bp-sequences of CO1 in *A. fimetarius* and *A. pedellus*. Of the columns, n_{ind} identifies the number of individuals successfully sequenced, and n_{hap} the number of unique haplotypes found among them, whereas V%, Pi% and S% report the fractions of sites which were variable, parsimony-informative and singletons, respectively.

Species	n_{ind}	n_{hap}	V%	Pi%	S%
A. fimetarius	31	17	3.47	2.45	1.02
A. pedellus	152 (39)	15 (7)	1.80 (1.09)	1.12 (0.43)	0.67 (0.65)

In *A. pedellus*, figures in brackets correspond to values obtained when removing the samples from Finland, thus allowing the explicit assessment of how this intensively-sampled region affects overall patterns (cf. Figs 2, 6).

This pattern holds true even when we remove the intensively sampled area of Finland (cf. Figs 2, 6) from the analyses, with the most frequently encountered haplotype still accounting for 77% of remaining samples.

The patterns of genetic diversity reported above seemed indicative of a recent range expansion within *A. pedellus*. The same inference was also supported by analyses of haplotype network structure: within *A. pedellus*, the haplotype network was found to be star-shaped, with multiple haplotypes separated by one or two mutations only. This contrasted with the shape of the *A. fimetarius* haplotype network, which displayed a more branched topology (Fig. 4). As a result, the mismatch distribution observed in *A. pedellus* fits expectations under Rogers' (1995) 'sudden expansion model' ($\chi^2 = 0.14$, df = 12, P = 1.0 for the full dataset; and $\chi^2 = 0.04$, df = 7, P = 1.0, when we remove all samples from Finland), whereas in *A. fimetarius*, it does not ($\chi^2 = 2193.55$, df = 7, P < 0.01; Fig. 7). Likewise, significant deviations from neutrality indicative of relatively recent population expansion events were detected by the Fu's F_s statistics for *A. pedellus* ($Fs = -9.70$, P < 0.01 using all samples of *A. pedellus*; $F_s = -3.1$, P = 0.01 when we remove the samples from Finland) but not for *A. fimetarius* ($F_s = -4.02$, P = 0.06).

Table 3. Morphological characters found to distinguish between the two 'cryptic' taxa *A. fimetarius* and *A. pedellus*. These characters are illustrated in Fig. 5.

	Aphodius fimetarius	*Aphodius pedellus*
Head	Lateral lobes ('genae') only slightly protruding (Fig. 5D–F); mostly very flat, semi-oval (with the strongest curvature in front of the middle, the posterior margin straight) (Fig. 5E–F); if a little more strongly protruding, anterior always more strongly convex than posterior with the greatest protrusion anterior to the middle, i.e. semi-parabolic (Fig. 5D, right side, arrow). Posterior portions of lobes, behind point of maximum width, parallel to one another or almost so.	Lateral lobes protruding (Fig. 5A–C); moderately to pronouncedly semicircular; if less protruding, then greatest protrusion close to the middle (Fig. 5C), i.e. semicircular or hyperbolic. Posterior portion of lobes, behind point of maximum width, more clearly convergent.
Elytral apex	Subapical area of elytra smooth and dull (Fig. 5J, K), sometimes finely reticulate, but without strong wrinkles or raised spots; rarely slightly uneven and/or with microscopic, dense, dull wrinkles or dull, slightly raised spots (Fig. 5L). Raised spots or wrinkles never shiny.	Subapical area of elytra more coarsely reticulate, shinier, with wrinkles, micro-wrinkles, or tiny raised shiny spots (Fig. 5H, I); microsculpture often shallow, but surface rarely smooth; if smooth, then not dull, but rather sparkly and uneven (Fig. 5G).
Elytral intervals	Apical end of elytral intervals more strongly convex (Fig. 5J–L), often with fourth interval extending to the tip (Fig. 5L, dot). Elytral intervals on average more strongly convex than in *pedellus*.	Apical end of elytral intervals flat (Fig. 5H), rarely with fourth interval extending up to the tip (Fig. 5I, dot).

In *A. pedellus*, common haplotypes centrally placed within the networks proved globally distributed, with no indication of phylogeographic structure. Haplotypes with affinities to less central clades were detected in widely different parts of Europe, with no suggestion of a local origin. The same pattern emerged within the intensively sampled area of Finland (Fig. 6). Here, individual populations of *A. pedellus* were characterized by high levels of haplotype polymorphism. Yet, no population differentiation was detected within this area, as most common haplotypes proved widespread within the country, and local variation in haplotype composition seemed more indicative of random sampling processes than of actual differentiation (Fig. 6). For *A. fimetarius*, inferences regarding potential population structuring were more constrained by limited sample size. Nonetheless, we notice that hardly any haplotypes are shared between the regions analysed (Fig. 6), suggesting some level of phylogeographic structuring in this species.

Do the species exhibit ecological differences when co-occurring?

Where examined within an area of sympatry in the Western US, *A. fimetarius* and *A. pedellus* showed a significant difference in phenology. In this strongly seasonal environment, the two species co-occurred during the earlier part of the summer, but *A. pedellus* exhibited a second peak in September, with no corresponding pattern in *A. fimetarius* (Fig. 8). This pattern was evident as a significant interaction between species identity and month (Table 4), against a backdrop of differences in the average abundance of the respective species (Table 4; main effect of Species), in the average abundance of both species across the two sites examined (Table 4; main effect of Site), in the specific abundance of the two species at the respective sites (Table 4; interaction Site × Species), and in overall dung beetle abundance across months (Table 4; main effect of Month and interaction Site × Month). Importantly, the difference on phenology was consistent across both sites (Table 4; note the non-significant three-way interaction Site × Month × Species).

Discussion

While some cryptic species may be virtually impossible to distinguish by external characters, our study reveals that karyotypes, DNA sequences and morphological traits offer consistent identification of the two cryptic taxa recently resolved within *A. fimetarius* sensu lato by karyotype criteria. Both taxa exhibit a worldwide distribution, with *A. pedellus* dominating in Northern parts of the globe. The two taxa were characterized by different patterns of genetic variation, suggesting different histories of the respective populations. Where sympatric, the taxa were found to exhibit ecological differences in the first (and so far only) trait examined. These findings all support the existence of two valid species within *A. fimetarius* sensu lato. Importantly, they also suggest that these two species may be characterized by different ecology and different range dynamics, and that resolving them may then help us understand the present, past and future of a numerically abundant dung beetle. Below, we will examine each finding in turn.

Are Aphodius fimetarius and A. pedellus valid species?

As recently as in 2000, *Aphodius fimetarius* sensu lato was still thought to form a single good species. Had Christine Wilson, then a PhD student, not screened '*Aphodius fimetarius*' from East Kent in addition to Berkshire material already karyotyped, the existence of two distinct taxa might still be unknown. The current study now offers unequivocal proof that the two

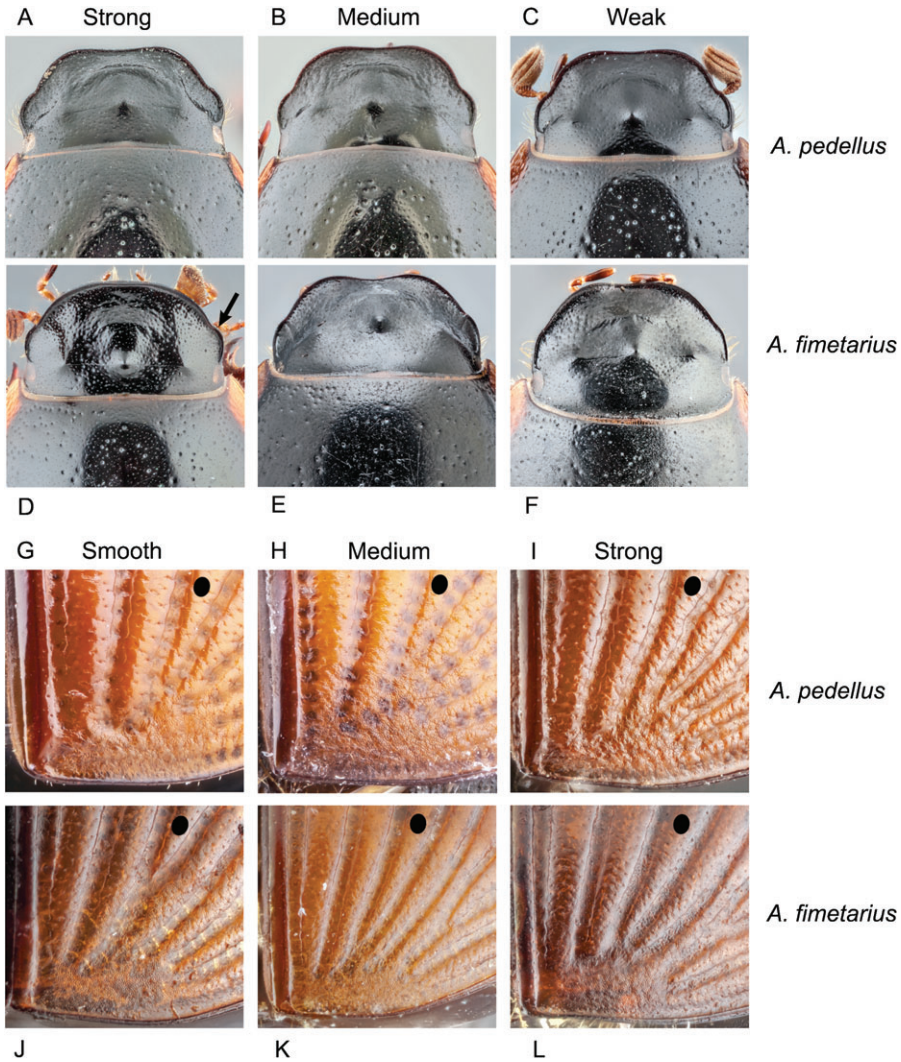

Fig. 5. Morphological variation within and between *Aphodius pedellus* and *A. fimetarius* (see Table 3). Individuals were chosen to represent the full range of variation. (A–F) Head, females: shape of the lateral lobes; strongly pronounced, medium, and weakly pronounced [for clarity, the structure referred to has been marked by an arrow in (D)]; (G–L) Elytra: microsculpture of subapical area; smooth, medium, and strongly developed (for clarity, the fourth elytral interval referred to in Table 3 has been marked by a black dot). (A) *A. pedellus* DMNS ZE.31761, Colorado, 10.viii.2012; (B) *A. pedellus* DMNS ZE.35095, Finland, 2008; (C) *A. pedellus* DMNS ZE.5662, Germany, 31.vii.1983; (D) *A. fimetarius* DMNS ZE.5660, Germany, 7.vii.1984; (E) *A. fimetarius* DMNS ZE.4115, Colorado, 23.v.2008; (F) *A. fimetarius* DMNS ZE.4805, Colorado, 22.vii.2009; (G) *A. pedellus*, DMNS ZE.35091, West Virginia, 25.vii.2000; (H) *A. pedellus*, DMNS ZE.35096, Finland, 19.vi.2011; (I) *A. pedellus* DMNS ZE.5659, Colorado, 19.ix.2010; (J) *A. fimetarius* DMNS ZE.4115, Colorado, 23.v.2008; (K) *A. fimetarius* DMNS ZE.4117, Colorado, 23.v.2008; (L) *A. fimetarius* DMNS ZE.4805, Colorado, 22.vii.2009. Photographs taken by Chris Grinter, DMNS.

species should be considered valid taxonomic entities, with characters of three different types all supporting a species-level split: Karyotypes, DNA sequences and morphological traits all attribute individuals to the same two clades, with less variation within than between them, and very little morphological overlap.

The current findings have three important implications. First, from the perspective of insect systematics, they support the division of the former composite species *A. fimetarius* into *A. fimetarius* sensu stricto and *A. pedellus*, firmly refuting any calls for the re-synonymisation of taxa (e.g. Bordat, 2002). Second, from the practical perspective of actually identifying these taxa, they suggest that characters of different types may be used interchangeably – and that for less typical individuals, different characters will usefully complement each other. Third and most practical of all, they suggest that for a large majority of specimens, morphological characters will suffice to arrive at species assignment. While this mode of identification is clearly the quickest and most straight-forward one, its use was only made possible by first detecting the two taxa by karyotypes, then validating the existence of morphological differences by other criteria.

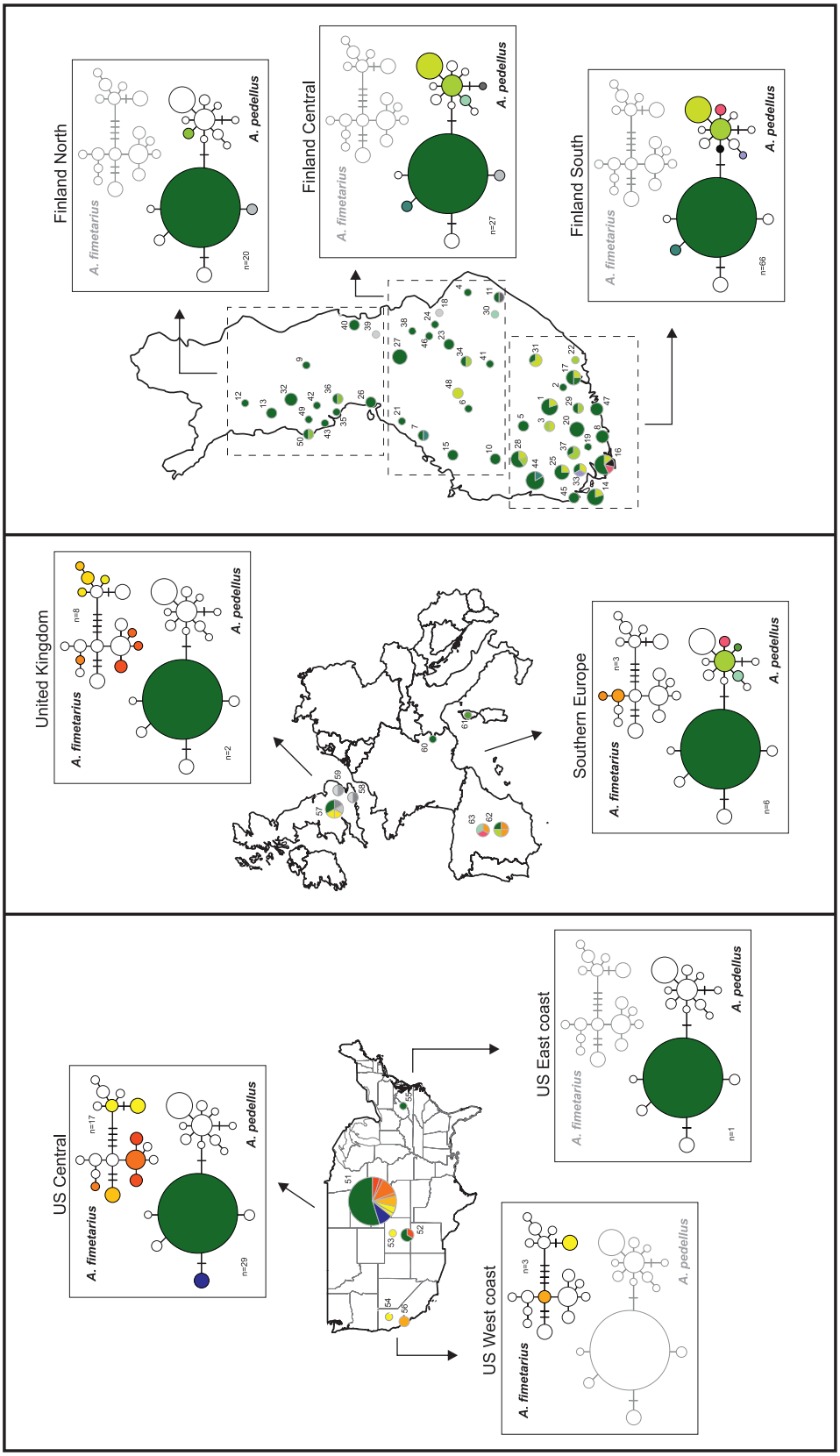

Fig. 6. Spatial distribution of haplotype diversity within *Aphodius fimetarius* and *A. pedellus*. Here, pie charts show haplotype frequencies for individual localities, with map insets showing compound frequencies within hierarchically larger geographic areas. Within these insets, individual haplotypes are mapped onto the general haplotype network introduced in Fig. 4, to match information on occurrence and frequency with information on phylogenetic relationships.

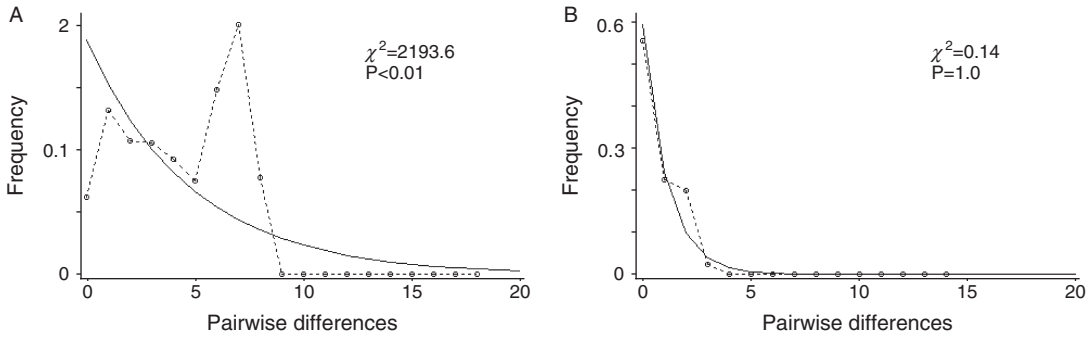

Fig. 7. Observed distributions of pairwise differences (dashed line) compared with the mismatch distribution expected under Rogers's (1995) 'sudden expansion model' (solid line) in (A) *Aphodius fimetarius* and (B) *A. pedellus*.

Overall, the history behind the description of two cryptic taxa in *A. fimetarius* shows how more complex characters may be used as heuristic tools in screening for cryptic species – and how, once such species limits have been uncovered, they may then guide systematists to detect differences in the morphology of such taxa (Wilson, 2001; Wilson & Angus, 2004; Yang *et al.*, 2012; Mutanen *et al.*, 2013). Traditionally, the opposite sequence of discovery has likely been more frequent, where morphological or ecological variation has lead researchers to hypothesize the existence of multiple species within presumptive species, then used more elaborate methods to demonstrate that this is indeed the case (e.g. Kaila & Albrecht, 1994; Audisio *et al.*, 2009).

Where do the two species occur?

Since the recognition of *A. pedellus*, it has now been reported from stray locations in England, France (and probably in eastern Siberia too, Wilson, 2001), with additional findings in Spain (Wilson & Angus, 2004), Italy, Slovakia and Slovenia (Whitehead, 2006). Individuals with a karyotype characteristic of *A. pedellus* have also been detected on Öland, Sweden (R. Angus, unpublished data), while Wilson (2001) interpreted Virkki's (Virkki, 1951) drawings of a Finnish karyotype as representing *A. pedellus*. What has still hampered the large-scale assessment of species-specific distributions has been the lack of unequivocal external characters. With only karyotyped individuals offering conclusive proof of the species' occurrence, the screening of general collections has been impossible – as karyotype preparations can only be obtained from live individuals, through a laborious process (Angus, 2006). Based on our cross-validation of different identification criteria, we may now combine materials identified by different characters – be they karyotypes, DNA sequences and/or morphological traits – to evaluate the global distribution of the two species.

While admittedly based on a finite material, our current assessment of major collections suggests both extensive sympatry and allopatry of the two taxa. Both taxa seem essentially holarctically distributed, with currently major sympatry within Central and Southern Europe and mixed patterns of sympatry within the US. Northern areas of Europe, Asia and North America are dominated by *A. pedellus* alone, confirming Rößner's (2012) initial suggestions.

While the current data shed light on where the two species occur, they will not suffice to establish how they got there. Nonetheless, to at least some parts of the world, *A. fimetarius* has apparently been introduced by human activities. To Australia, *A. fimetarius* was brought accidentally (Tyndale-Biscor, 1990). Rößner (2012) erroneously reported an introduction of *A. pedellus* to South America (E. Rößner, personal communication) where neither *A. fimetarius* nor *A. pedellus* occur (Skelley *et al.*, 2007). In America, the range of *Aphodius fimetarius* sensu lato extends southward to the State of Puebla in east-central Mexico (Navarrete-Heredia, 2006), but the species identity of these records has yet to be determined.

When do the two species occur?

The extent to which *A. fimetarius* and *A. pedellus* differ in their ecology remains poorly explored. In the current context, we only examined a single trait which was easy to score, i.e. the activity period of the two species within a single area of co-occurrence. Here, a distinct difference in phenology was observed among the two species: while both species were active during the early part of the summer, only *A. pedellus* exhibited a second peak in abundance during the early autumn, when *A. fimetarius* remained inactive. This was no spurious finding, but a pattern repeated across two sites and multiple years (Table 4, Fig. 8). Clearly, this specific phenological pattern should not be generalized to the whole species, nor is it necessarily repeated across latitudes or longitudes. What it does show is that in at least one strongly seasonal environment, the two species show different life cycles. This ecological difference adds to the distinctness of the two taxa, and suggests that they may prove to differ in more traits once examined. Differentiating between the two species, and exploring their ecology in more detail, is then a priority for understanding both the natural history and the population dynamics of this abundant and widespread dung beetle.

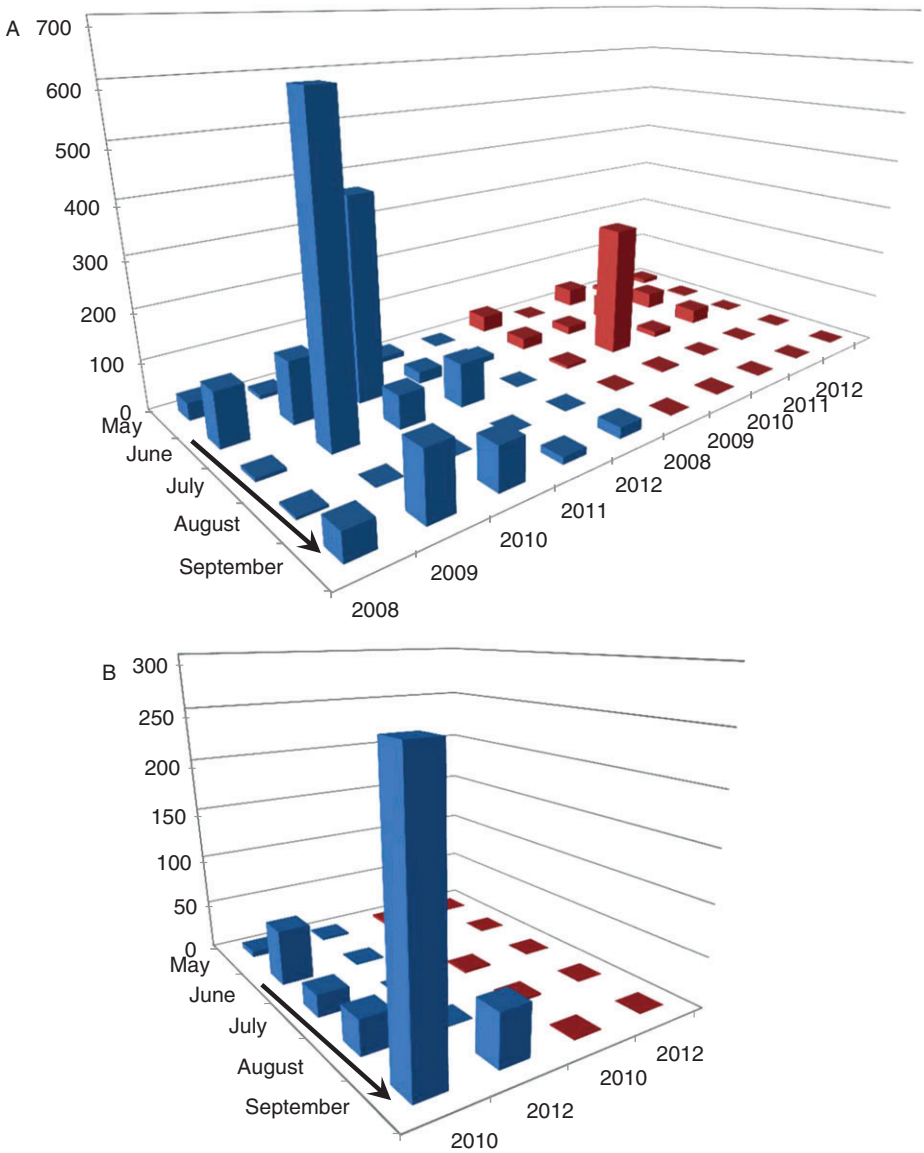

Fig. 8. Phenology of *Aphodius pedellus* (blue) and *A. fimetarius* (red) at Bijou Creek (A) and Keen Ranch (B) in Colorado, U.S.A. Shown is the number of individuals caught in a standard sample taken each month from May to September during multiple years (four for Bijou Creek, two for Keen Ranch).

Different population histories in Aphodius fimetarius and A. pedellus?

Patterns of genetic diversity within *A. pedellus* offered signs of a recent population expansion (cf. Hanski *et al.*, 2008), whereas patterns in *A. fimetarius* seemed more indicative of an old and stable – or indeed a declining population (Rogers & Harpending, 1992; Rogers, 1995; Ramírez-Soriano *et al.*, 2008). In given parts of the world, recent expansions may either reflect anthropogenic introductions or more gradual natural change – but at least for Europe, consistently lower haplotype diversity with increasing latitude (cf. Fig. 6) seems to mimic the range expansion of many other taxa following the retreat of the last ice age (Hewitt, 2000, 2004). In North America, the low diversity observed in *A. pedellus* may suggest either a recent colonization of the continent by *A. pedellus*, or other historical bottlenecks in population size.

While the exact mechanisms behind the patterns observed here cannot be conclusively established, they do offer fuel for an interesting hypothesis: perhaps in recent history, one cryptic species has been sweeping through the world at the expense of another, as perhaps aided by anthropogenic introductions and/or climate shifts to its particular favour. Such an interpretation is also supported by the currently more fragmentary distribution of *A. fimetarius*, as contrasting with the evidently wide distribution of *A. pedellus* (Fig. 2). Our hypothesis may well be premature,

Table 4. Generalized linear model of species abundances at two farms, Bijou Creek and Keen Ranch in Colorado, U.S.A. Shown are likelihood ratio statistics for Type 3 analysis.

Source	DF	χ^2	P
Species	1	190.45	< 0.0001
Site	1	30.09	< 0.0001
Month	4	64.14	< 0.0001
Site × species	1	11.01	0.0009
Site × month	4	47.78	< 0.0001
Month × species	4	80.01	< 0.0001
Site × month × species	4	3.51	0.48

but the characters developed in our study now offer the tools for critically assessing it. We urge our colleagues to help us compile the material for a comprehensive assessment of the global distribution of the two species, and of recent changes to it.

Conclusions

In 2000, *Aphodius fimetarius* was still thought to form a single valid species. This notion was challenged by the proposal of two distinct taxa, as distinguishable by hard-scored chromosomal characters. The current study now puts the existence of the two cryptic taxa beyond doubt, and shows how they may be identified by multiple and mutually consistent and thereby complementary criteria. It also suggests that these two species may be characterized by different ecology and by different dynamics across the globe. At this stage, we should remind ourselves that *A. fimetarius* and *A. pedellus* have a vast distribution across the globe (current study), and are locally highly abundant (Gordon & Skelley, 2007; Roslin & Heliövaara, 2009). If such an insect taxon hides two cryptic species – then what other surprises might systematic entomology have in store for us?

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12079

Table S1. List of all material examined, with specimen identification, locality of specimens, specimens vouchers, species identification using chromosomal preparations, mitochondrial COI haplotype (with respective GenBank accession number) and morphology. Collections examined: The Natural History Museum, London (BMNH); the Denver Museum of Nature & Science, Denver, Colorado (DMNS); the Field Museum of Natural History, Chicago (FMNH); The Hasbrouck Insect Collection at Arizona State University, Tempe, Arizona (HICASU); the private collections of C. J. Wilson (CJW) and Robert Angus, London (RBA); and some specimens from the C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins (CSU), the Erster Vorarlberger Coleopterologischer Verein, Bürs, Austria (EVCV); and the National Museum of Ireland in Dublin (NMID).

Acknowledgements

We would like to thank Hetta-Mari and Atte Rajapuro for assisting in fieldwork across Finland; Tudi Arneill, Sylvia Krell-Westerwalbesloh, and Jeffrey Stephenson for help in the field in Colorado; Mikko Pentinsaari for kindly providing additional samples, and all members of the Spatial Foodweb Ecology Group for general support on innumerable occasions. Mattias Forshage and Jason Maté provided important samples and insightful advice during all parts of this work. Eckehard Rößner, Schwerin, shared the results of his morphological screening of extensive material. Financial support from the Academy of Finland (grant number 138346 to T. Roslin), from Prairie Biotic Research, Inc. (to FTK) and from the Entomological Society of Finland, the Entomological Society of Helsinki, the Kuopio Naturalists' Society, and Societas pro Fauna et Flora Fennica (to MS) is gratefully acknowledged.

References

Angus, R.B. (2006) Chromosome differences. *A Coleopterist's Handbook*, 4th edn (ed. by J. Cooter and M.V.L. Barclay), pp. 346–351. Amateur Entomologists' Society, Orpington, Kent.

Angus, R.B., Wilson, C.J. & Krell, F.-T. (2012) Case 3579. *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae): proposed conservation of usage of the specific name by designation of a neotype. *Bulletin of Zoological Nomenclature*, **69**, 29–36.

Ashrafi, S., Bontadina, F., Kiefer, A., Pavlinic, I. & Arlettaz, R. (2010) Multiple morphological characters needed for field identification of cryptic long-eared bat species around the Swiss Alps. *Journal of Zoology*, **281**, 241–248.

Audisio, P., Brustel, H., Carpaneto, G.M., Coletti, G., Mancini, E., Trizzino, M., Antonini, G. & De Biase, A. (2009) Data on molecular taxonomy and genetic diversification of the European Hermit beetles, a species complex of endangered insects (Coleoptera: Scarabaeidae, Cetoniinae, *Osmoderma*). *Journal of Zoological Systematics and Evolutionary Research*, **47**, 88–95.

Baker, R.J. & Bradley, R.D. (2006) Speciation in mammals and the genetic species concept. *Journal of Mammalogy*, **87**, 643–662.

Ballerio, A. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (5). *Bulletin of Zoological Nomenclature*, **69**, 227–228.

Barclay, M.V.L. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (5). *Bulletin of Zoological Nomenclature*, **69**, 139–140.

Barratt, E.M., Deaville, R., Burland, T.M., Bruford, M.W., Jones, G., Racey, P.A. & Wayne, R.K. (1997) DNA answers the call of pipistrelle bat species. *Nature*, **387**, 138–139.

Bellmann, A., Hillert, O. & Rößner, E. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (2). *Bulletin of Zoological Nomenclature*, **69**, 136–138.

Bezdek, A. & Král, D. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (1). *Bulletin of Zoological Nomenclature*, **69**, 284.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K. & Das, I. (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, **22**, 148–155.

Bordat, P. (2002) A propos de quelques espèces d'Aphodiens de la faune de France. *Coléoptériste*, **5**, 129–130.

Branco, T. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (6). *Bulletin of Zoological Nomenclature*, **69**, 228–229.

Bucklin, A., Steinke, D. & Blanco-Bercial, L. (2011) DNA barcoding of marine metazoa. *Annual Review of Marine Science*, **3**, 471–508.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1659.

Colborn, J., Crabtree, R.E., Shaklee, J.B., Pfeiler, E. & Bowen, B.W. (2001) The evolutionary enigma of bonefishes (*Albula* spp.): cryptic species and ancient separations in a globally distributed shorefish. *Evolution*, **55**, 807–820.

Costa, F.O. & Carvalho, G.R. (2010) New insights into molecular evolution: prospects from the Barcode of Life Initiative (BOLI). *Theory in Biosciences*, **129**, 149–157.

Dellacasa, M. & Dellacasa, G. (2003) Review of the genus *Aphodius* (Coleoptera: Aphodiidae). *Folia Heyrovskyana*, **11**, 173–202.

Dellacasa, M. & Dellacasa, G. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (1). *Bulletin of Zoological Nomenclature*, **69**, 222–223.

Detwiler, J.T., Bos, D.H. & Minchella, D.J. (2010) Revealing the secret lives of cryptic species: examining the phylogenetic relationships of echinostome parasites in North America. *Molecular Phylogenetics and Evolution*, **55**, 611–620.

Dlugosch, K.M. & Parker, I.M. (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Drummond, A., Ashton, B., Buxton, S. *et al.* (2011) *Geneious v.5.4* [WWW document]. URL http://www.geneious.com [accessed on 12 February 2013].

Excoffier, L. & Lischer, H.E. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Excoffier, L., Foll, M. & Petit, R.J. (2009) Genetic consequences of range expansions. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 481–501.

Falahee, S.L. & Angus, R.B. (2010) Chromosomal separation of difficult species of *Copris* Geoffroy, 1762 and *Onthophagus* Latreille, 1802 (Coleoptera, Scarabaeidae), with discussion of *O. massai* Baraud as a British Pleistocene fossil. *Zookeys*, **34**, 17–32.

Fery, H. (2012a) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (1). *Bulletin of Zoological Nomenclature*, **69**, 128–136.

Fery, H. (2012b) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (4). *Bulletin of Zoological Nomenclature*, **69**, 291–293.

Fery, H. (2013) Comment on the proposed conservation of usage of the specific name *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579). *Bulletin of Zoological Nomenclature*, **70**, 48–51.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Fontaneto, D., Iakovenko, N., Eyres, I., Kaya, M., Wyman, M. & Barraclough, T.G. (2011) Cryptic diversity in the genus *Adineta* Hudson & Gosse, 1886 (Rotifera: Bdelloidea: Adinetidae): a DNA taxonomy approach. *Hydrobiologia*, **662**, 27–33.

Forshage, M. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (2). *Bulletin of Zoological Nomenclature*, **69**, 223–224.

Frolov, A. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, SCARABAEIDAE) by designation of a neotype (Case 3579) (3). *Bulletin of Zoological Nomenclature*, **69**, 225.

Fu, Y.X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Funk, W.C., Caminer, M. & Ron, S.R. (2012) High levels of cryptic species diversity uncovered in Amazonian frogs. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1806–1814.

Glaw, F. & Vences, M. (2002) A new cryptic frog species of the *Mantidactylus boulengeri* group with a divergent vocal sac structure. *Amphibia-Reptilia*, **23**, 293–304.

Gordon, R.D. & Skelley, P.E. (2007) *A Monograph of the Aphodiini Inhabiting the United States and Canada (Coleoptera: Scarabaeidae: Aphodiinae)*. Memoirs of the American Entomological Institute Vol. 79, Gainesville, Florida.

Hanski, I., Wirta, H., Nyman, T. & Rahagalala, P. (2008) Resource shifts in Malagasy dung beetles: contrasting processes revealed by dissimilar spatial genetic patterns. *Ecology Letters*, **11**, 1208–1215.

Hausmann, A., Hebert, P.D.N., Mitchell, A., Rougerie, R., Sommerer, M., Edwards, T. & Young, C.J. (2009) Revision of the Australian *Oenochroma vinaria* Guenée, 1858 species-complex (Lepidoptera: Geometridae, Oenochrominae): DNA barcoding reveals cryptic diversity and assesses status of type specimen without dissection. *Zootaxa*, **2239**, 1–21.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & DeWaard, J.R. (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 313–321.

Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R. (2003b) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B: Biological Sciences*, **270**, S96–S99.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 14812–14817.

Hewitt, G.M. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hewitt, G.M. (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences*, **359**, 183–195.

Kaartinen, R., Stone, G.N., Hearn, J., Lohse, K. & Roslin, T. (2010) Revealing secret liaisons: DNA barcoding changes our understanding of food webs. *Ecological Entomology*, **35**, 623–638.

Kaila, L. & Albrecht, A. (1994) The classification of the *Timandra griseata* group (Lepidoptera: Geometridae, Sterrhinae). *Insect Systematics & Evolution*, **25**, 461–479.

Krell, F.-T. (2007) Dung Beetle Sampling Protocols. *Denver Museum of Nature & Science Technical Report*, **2007–6**, 1–11.

Krell, F.-T. & Angus, R.B. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (3). *Bulletin of Zoological Nomenclature*, **69**, 285–290.

Kyle, C.J., Johnson, A.R., Patterson, B.R., Wilson, P.J., Shami, K., Grewal, S.K. & White, B.N. (2006) Genetic nature of eastern wolves: past, present and future. *Conservation Genetics*, **7**, 273–287.

Lumley, L.M. & Sperling, F.A.H. (2010) Integrating morphology and mitochondrial DNA for species delimitation within the spruce budworm (*Choristoneura fumiferana*) cryptic species complex (Lepidoptera: Tortricidae). *Systematic Entomology*, **35**, 416–428.

Marjoram, P. & Donnelly, P. (1994) Pairwise comparisons of mitochondrial-DNA sequences in subdivided populations and implications for early human-evolution. *Genetics*, **136**, 673–683.

Maté, J. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (4). *Bulletin of Zoological Nomenclature*, **69**, 225–227.

Meyer, C.P. & Paulay, G. (2005) DNA barcoding: error rates based on comprehensive sampling. *PLoS Biology*, **3**, e422.

Murray, D.L. & Waits, L.P. (2007) Taxonomic status and conservation strategy of the endangered red wolf: a response to Kyle et al. (2006). *Conservation Genetics*, **8**, 1483–1485.

Mutanen, M., Kaila, L. & Tabell, J. (2013) Wide-ranging barcoding aids discovery of one-third increase of species richness in presumably well-investigated moths. *Scientific Reports*, **3**, 2901. DOI: 10.1038/srep02901.

Navarrete-Heredia, J.L. (2006) Notes on three adventive species of Coleoptera (Hydrophilidae and Scarabaeidae) from Baja California, with additional data from other Mexican states. *Entomological News*, **117**, 211–218.

Padial, J., Miralles, A., De la Riva, I. & Vences, M. (2010) The integrative future of taxonomy. *Frontiers in Zoology*, **7**, 16. DOI: 10.1186/1742-9994-7-16.

Ramírez-Soriano, A., Ramos-Onsins, S.E., Rozas, J., Calafell, F. & Navarro, A. (2008) Statistical power analysis of neutrality tests under demographic expansions, contractions and bottlenecks with recombination. *Genetics*, **179**, 555–567.

Rößner, E. (2012) *Die Hirschkäfer und Blatthornkäfer (Coleoptera: Scarabaeoidea) Ostdeutschlands*. Verein der Freunde und Förderer des Naturkundemuseums Erfurt, Erfurt.

Rogers, A.R. (1995) Genetic-evidence for a pleistocene population explosion. *Evolution*, **49**, 608–615.

Rogers, A.R. & Harpending, H. (1992) Population-growth makes waves in the distribution of pairwise genetic-differences. *Molecular Biology and Evolution*, **9**, 552–569.

Roslin, T. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (3). *Bulletin of Zoological Nomenclature*, **69**, 138.

Roslin, T. & Heliövaara, K. (2009) *Suomen lantakuoriaiset. Opas santiaisista lantiaisiin. [Dung Beetles of Finland]*. Gaudeamus, Helsinki.

Schmidt, L., Kalz, H., Schulze, J., Schulze, W., Ziegler, W. & Zorn, C. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (Case 3579) (2). *Bulletin of Zoological Nomenclature*, **69**, 285.

Skelley, P.E., Dellacasa, M., Dellacasa, G. & Gordon, R.D. (2007) Checklist of the Aphodiini of Mexico, Central and South America (Coleptera: Scarabaeidae: Aphodiinae). *Insecta Mundi*, **14**, 1–14.

Solodovnikov, A. (2012) Comments on the proposed conservation of usage of the specific name of *Scarabaeus fimetarius* Linnaeus, 1758 (currently *Aphodius fimetarius*; Insecta, Coleoptera, Scarabaeidae) by designation of a neotype (4). *Bulletin of Zoological Nomenclature*, **69**, 138.

Teacher, A.G.F. & Griffiths, D.J. (2011) HapStar: automated haplotype network layout and visualization. *Molecular Ecology Resources*, **11**, 151–153.

Templeton, A.R., Crandall, K.A. & Sing, C.F. (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA-sequence sata. 3. Cladogram estimation. *Genetics*, **132**, 619–633.

Thabah, A., Rossiter, S.J., Kingston, T., Zhang, S., Parsons, S., Mya, K.M., Akbar, Z. & Jones, G. (2006) Genetic divergence and echolocation call frequency in cryptic species of *Hipposideros larvatus* s.l. (Chiroptera: Hipposideridae) from the Indo-Malayan region. *Biological Journal of the Linnean Society*, **88**, 119–130.

Tyndale-Biscor, M. (1990) *Common Dung Beetles in Pastures of South-eastern Australia*. CSIRO Division of Entomology, Canberra.

Valentini, A., Pompanon, F. & Taberlet, P. (2009) DNA barcoding for ecologists. *Trends in Ecology & Evolution*, **24**, 110–117.

Virkki, N. (1951) Zur Zytologie einiger Scarabaeiden (Coleoptera). Studien an der Spermatogenese. *Annales Zoologici Societatis Zoologicae-Botanicae Fennicae "Vanamo"*, **14**, 1–105.

Wakeley, J. & Hey, J. (1997) Estimating ancestral population parameters. *Genetics*, **145**, 847–855.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. & Hebert, P.D.N. (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, **360**, 1847–1857.

Waugh, J. (2007) DNA barcoding in animal species: progress, potential and pitfalls. *BioEssays*, **29**, 188–197.

Whitehead, P.F. (2006) *Aphodius (A.) fimetarius* (L., 1758) and *Aphodius (A.) pedellus* (DeGeer, 1774) (Col., Aphodiidae) are distinct species with new evidence for their European distribution. *Entomologists' Monthly Magazine*, **142**, 85–86.

Wilson, C.J. (2001) *Aphodius pedellus* (DeGeer), a species distinct from *A. fimetarius* (Linnaeus) (Coleoptera: Aphodiidae). *Tijdschrift voor Entomologie*, **144**, 137–143.

Wilson, C.J. & Angus, R.B. (2004) A chromosomal analysis of the West European species of *Aphodius* Illiger, subgenus *Aphodius* s. str. (Coleoptera: Aphodiidae). *Tijdschrift voor Entomologie*, **147**, 259–264.

Yang, Z., Landry, J.-F., Handfield, L. *et al.* (2012) DNA barcoding and morphology reveal three cryptic species of *Anania* (Lepidoptera: Crambidae: Pyraustinae) in North America, all distinct from their European counterpart. *Systematic Entomology*, **37**, 686–705.

Accepted 1 March 2014