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Hidden species within the genus *Ocys* Stephens: the widespread species *O. harpaloides* (Audinet-Serville) and *O. tachysoides* (Antoine) (Coleoptera, Carabidae, Bembidiini)

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Abstract

Beetles previously considered to be *Ocys harpaloides* (Audinet-Serville) from northern Africa, Spain, France, the United Kingdom, France, and Belgium belong to two species. These species can be distinguished using DNA sequences of 28S rDNA, 18S rDNA, COI, CAD, and topoisomerase I. A key, diagnoses, and images are provided to enable identification of specimens based upon characteristics of male and female genitalia, as well as microsculpture and other external structures. Through examination of the holotype of *Bembidium harpaloides* v. *tachysoides* Antoine, as well as designation of lectotypes of *Bembidion harpaloides* Audinet-Serville and *Ocys melanocephalus* Stephens, and designation of a neotype for *Tachis rufescens* Guérin-Méneville, the valid names of the two species were determined to be *Ocys harpaloides* and *Ocys tachysoides* (Antoine).

Introduction

Among European carabid beetles of the tribe Bembidiini Stephens, *Ocys harpaloides* (Audinet-Serville, 1821) is one of the more distinctive, looking more similar to members of other tribes (for example, the trechine *Trechus* Clairville, 1806 or the tachyine *Porotachys* Netolitzky, 1914) than to other bembidiines. As currently circumscribed, *Ocys harpaloides* is a common and widespread Mediterranean-Atlantic species, ranging from the Azores, Algeria and Morocco north to southern Norway, Britain and Ireland, and east to Italy, the Balkans and Greece (Lindroth 1992).

The only congener with which *O. harpaloides* is documented to coexist throughout much of this range is *Ocys quinquestriatus* (Gyllenhal), a species from which it can easily be distinguished by numerous morphological characters (Lindroth 1974; see below). There are more than 20 additional species of *Ocys* Stephens; almost all of these are rare, and restricted to small geographic areas (e.g., Giachino and Vailati 2012; Magrini and Degiovanni 2009; Neri et al. 2011; Toledano and Wrase 2016; Vigna Taglianti 1994). Many of these distinctive, localized endemics occur at higher elevations in mountains in Spain, northern Africa, or Italy (Magrini and Degiovanni 2009; Neri, et al. 2011; Netolitzky 1942), or on islands (Magrini et al. 1998). However, one species similar to *O. harpaloides*, *Ocys tachysoides* (Antoine, 1933), has been reported from low elevation habitats in Morocco (Antoine 1933; 1955), Spain (Toribio 2013), and a montane region of Portugal (Sciaky 1998).

In Northern Ireland, two morphologically distinct forms of "*Ocys harpaloides*", correlated with habitat, have been reported (Anderson et al. 2000): specimens found among

Figure 1. Habitats. **A**: East of Colliery Bay, Fair Head, Northern Ireland; **B**: Hollymount NNR near Downpatrick, Northern Ireland.

coastal rocks (Fig. 1A) are paler and larger (Fig. 2A), and those found in woodlands (Fig. 1B) are darker and smaller (Fig. 2B) (Anderson et al. 2000). Although this pattern might be a result of ecophenotypic variation, the observation suggested the possibility that two species could be hiding within "*Ocys harpaloides*". However, initial morphological investigations did not reveal additional, definitive differences (Anderson et al. 2000).

In 2011 we began an examination of the DNA sequences of these forms, and more detailed study of morphological variation. This revealed that two species were indeed present in Northern Ireland, and elsewhere in the range of "*Ocys harpaloides*". As this paper was being prepared for publication, Raupach et al. (2016) reported the existence of two forms of "*Ocys harpaloides*" in Germany and France based upon the cytochrome oxidase I gene; these two forms correspond to the two species detailed here. In this paper, we document the differences between the two species, and through examination of types of the available names, show that the valid name of the second species is *Ocys tachysoides* (Antoine), which is much more widespread than previously believed.

Methods

Members of *Ocys* were examined from the collections listed below. Each collection's listing begins with the coden used in the text.

BMNH The Natural History Museum, London
CTVR Luca Toledano Collection, Verona, Italy
DE Dominique Echaroux Collection, Etréchy, France
MNHN Muséum National d'Histoire Naturelle, Paris
OSAC Oregon State Arthropod Collection, Oregon State University
PB Paolo Bonavita Collection, Rome, Italy
RAC Roy Anderson Collection, Belfast, United Kingdom

Morphological methods. General methods of specimen preparation for morphological work, and terms used, follow Maddison (1993; 2008). Genitalia were prepared, after dissection from the body, by treatment in 10% KOH at 65 °C for 10 minutes followed by multi-hour baths of distilled water, 5% glacial acetic acid, distilled water, and then ethanol. Male genitalia were then mounted in Euparal between two small coverslips attached to archival-quality heavyweight watercolour paper, and, once dried, pinned beneath the specimen. For all type specimens except that of *Ocys melanocephalus* Stephens, genitalia were removed from Euparal through soaking in 100% ethanol, placed in small plastic vials containing glycerol, and pinned beneath the specimen.

Photographs of male genitalia were taken with a Leica Z6 and JVC KY-F75U camera using Microvision's Cartograph software for extended depth of field (EDF) processing; the images thus potentially have some artifacts caused by the EDF algorithm.

The following measurements were made:

Length: body length, from apex of the labrum to apex of the longer elytron.

GCR: gonocoxite ratio = gonocoxite 2 length / gonocoxite 1 length (Fig. 4)

Measurements were made either using Microvision's Cartograph software processing images from a JVC KY-F75U camera on a Leica Z6 lens, or on a Leica M3Z stereomicroscope with a Leitz graticule.

The density of the microsculpture lines on the left elytron was measured by counting the number of microsculpture lines that cross a 0.1 mm longitudinal line placed in the centre of the third elytral interval approximately 1/3 of the distance between the front and back of the elytron.

Taxon sampling for DNA studies. We obtained DNA sequence data for 15 *Ocys* specimens (Table 1), and combined these with results from the two *Ocys* specimens sequenced in Maddison (2012). For outgroups, we chose two or more species representing different lineages within each of the five largest genera of Bembidiina (*Bembidion* Latreille, *Asaphidion* Chaudoir, *Amerizus* des Gozis, *Sinechostictus* Motschulsky, and *Lionepha* Casey (Maddison, 2012)). The outgroup species are listed in Table 2.

DNA sequencing. Genes studied, and abbreviations used in this paper, are: **28S**: 28S ribosomal DNA (D1-D3 domains); **18S**: 18S ribosomal DNA (near full-length); **COI**: cytochrome oxidase I; **CAD**: carbamoyl phosphate synthetase domain of the *rudimentary* gene; **Topo**: topoisomerase I. In addition, sequences of the *wingless* gene and arginine kinase were acquired for specimens *Ocys harpaloides* 2759 and *Ocys tachysoides* 2758.

For all except specimen number 4606, DNA was extracted from specimens preserved in 95% ethanol using a Qiagen DNeasy Blood and Tissue Kit. Fragments for the seven genes were amplified using the Polymerase Chain Reaction on an Eppendorf Mastercycler Pro Thermal Cycler, using TaKaRa Ex Taq and the basic protocols recommended by the manufacturers. Primers and details of the cycling reactions used are given in Maddison (2012). The amplified products were then purified, quantified, and sequenced at the University of Arizona's Genomic

Table 1. Locality details for sequenced *Ocys* exclusive of *O. quinquestriatus*. Four-digit numbers at the start of each row are D.R. Maddison DNA voucher numbers. Specimen 0569 had been previously sequenced by Maddison (2012).

Ocys harpaloides	
2760	**Northern Ireland**: Colliery Bay, Ballycastle, Antrim, 55.2116°N 6.1910°W. 5.vii.2011. At grass roots on slope above high water mark. R. Anderson
2762	**Northern Ireland**: Colliery Bay, Ballycastle, Antrim, 55.2116°N 6.1910°W. 5.vii.2011. At grass roots on slope above high water mark. R. Anderson
2759	**Ireland**: Wexford, North Slob. 52.3546°N 6.4082°W. 20.vii.2011. Under driftwood on sandy beach. R. Anderson
0569	**Belgium**: Schorisse, Bos Ter Rijst. Approximately 50.78°N 3.69°E. 17.iv.1997. Temperate broadleaf forest. Konjev Desender
2937	**Belgium**: Schorisse, Bos Ter Rijst. Approximately 50.78°N 3.69°E. 17.iv.1997. Temperate broadleaf forest. Konjev Desender
2853	**Belgium**: Halve Maan, Oostende. 51.237°N 2.932°E. 23.ix.1995. Coastal salt marsh (non tidal). K. Desender.
4983	**Spain:** Sitges. 41.2383°N 01.8244°E. 9.ix.2006. W Maddison.
Ocys tachysoides	
2758	**Northern Ireland**: Belfast, Belvoir Forest. 54.5544°N, 5.9404°W. 10.iii.2011. Under bark dead alder and willow. R. Anderson
2761	**Northern Ireland**: Belfast, Belvoir Forest. 54.5544°N, 5.9404°W. 10.iii.2011. Under bark dead alder and willow. R. Anderson
2763	**Northern Ireland**: Belfast, Belvoir Forest. 54.5544°N, 5.9404°W. 10.iii.2011. Under bark dead alder and willow. R. Anderson
2898	**Northern Ireland**: Co. Down, Mount Stewart. 54.5491°N 5.6037°W. 12.x.2011. Under bark of sycamore/cedar/pine by sea. R. Anderson
2899	**Northern Ireland**: Co. Down, Murlough NNR. 54.2461°N 5.8330°W. 15.xi.2011. Under bark of sycamore, sandy wood near sea. R. Anderson
2936	**Belgium**: Moerzeke, De Kramp. Approximately 51.06°N 4.15°E. 1.iii.1999. Tidal freshwater marsh along the River Schelde. Konjev Desender
2938	**Belgium**: Kastel, Gespoelde. Approximately 51.05°N 4.16°E. 11.ix.1998 Tidal freshwater marsh along the River Schelde. Konjev Desender
4606	**Germany**: Nordrhein-Westfalen, Landkreis Unna, Selm-Bork, Lippeaue. 51.6533°N 7.4442°E. Under bark of *Salix*. K. Hannig

Table 2. Sampling of Bembidiina species. Four-digit numbers in the "#" column entries for *Ocys* are D.R. Maddison voucher numbers for sequenced specimens. Data for all non-*Ocys* specimens, as well as to specimens 1077 and 0569, were obtained from GenBank from previous studies (Maddison 2008; 2012; Maddison et al. 1999; Maddison and Ober 2011; Maddison and Swanson 2010; Wild and Maddison 2008), and more data about the specimens are presented in those papers. Locality details for *Ocys* specimens are given in Table 1 and in Maddison (2012). For each gene, GenBank accession numbers are listed.

	#	COI	28S	18S	CAD	Topo
Bembidion chalceum Dejean		EF649200	EF648892	EF648647	EF649431	EU677650
Bembidion rapidum (LeConte)		JN171095	EU677690	JN170224	EU677543	EU677642
Bembidion transversale Dejean		GU454797	EU677688	JN170242	EU677541	EU677639
Bembidion variegatum Say		JN171131	JN170458	JN170245	JN170937	JN171310
Asaphidion curtum (Heyden)		JN170977	GU556078	AF002792	JN170736	JN171163
Asaphidion yukonense Wickham		JN170979	JN170273	JN170139	EU677540	EU677638
Amerizus wingatei (Bland)		JN170974	JN170267	JN170136	JN170732	JN171160
Amerizus (Tiruka) sp.		JN170972	JN170265	JN170134	JN170730	JN171158
Sinechostictus elongatus (Dejean)		JN171152	JN170479	JN170260	JN170965	JN171332
Sinechostictus (Pseudolimnaeum) sp. 3		JN171150	JN170474	JN170259	JN170960	JN171329
Lionepha erasa (LeConte)		JN171141	JN170468	JN170252	JN170948	JN171320
Lionepha osculans (Casey)		JN171143	JN170470	JN170254	JN170950	JN171322
Lionepha disjuncta (Lindroth)		JN171142	JN170469	JN170253	JN170949	JN171321
Ocys quinquestriatus (Gyllenhal)	1077	JN171145	JN170472	JN170257	JN170954	JN171324
Ocys harpaloides (Audinet-Serville)	0569		GU556103	JN170256	JN170953	
	2759	KX907141	KX907154	KX907168	KX907176	KX907188
	2760	KX907142	KX907155		KX907177	KX907189
	2762	KX907143	KX907156		KX907178	KX907190
	2853	KX907148	KX907161		KX907183	KX907195
	2937	KX907152	KX907165			KX907199
	4983	KX907153	KX907166			
Ocys tachysoides (Antoine)	2758	KX907144	KX907157	KX907169	KX907179	KX907191
	2761	KX907145	KX907158		KX907180	KX907192
	2763	KX907146	KX907192		KX907181	KX907193
	2898	KX907147	KX907160		KX907182	KX907194
	2899	KX907151	KX907164			KX907198
	2936	KX907149	KX907162		KX907184	KX907196
	2938	KX907150	KX907163		KX907185	KX907197
	4606	KX907187	KX907175	KX907172	KX907186	KX907167

and Technology Core Facility using a 3730 XL Applied Biosystems automatic sequencer. Assembly of multiple chromatograms for each gene fragment and initial base calls were made with Phred (Green and Ewing 2002) and Phrap (Green 1999) as orchestrated by Mesquite's Chromaseq package (Maddison and Maddison 2014; Maddison and Maddison 2015b) with subsequent modifications by Chromaseq and manual inspection. Multiple peaks at a single position in multiple reads were coded using IUPAC ambiguity codes.

For specimen 4606, RNA was extracted from an RNA-Later preserved specimen using a Qiagen RNeasy Kit and Trizol (Life Technologies). Isolation of mRNA was done with an NEBNext® Poly(A) mRNA Magnetic Isolation Module (New England BioLabs). The library was prepared using an NEBNext Ultra RNA (New England BioLabs) kit using 1000 ng of input RNA and NEBNext Multiplex Oligos for Illumina (New England BioLabs). The finished library was quantified using a Qubit Florometer (Life Technologies) and fragment distribution characterized on a 2100 Bioanalyzer (Agilent Technologies). The library was then sequenced on an Illumina 2500 at the Oregon Health Sciences University's Massively Parallel Sequencing Shared Resource, with reads demultiplexed using CASAVA v1.8. The 100-base, paired-end run produced 36 million reads, which were assembled using CLC Genomics Workbench version 8.5, using the *de novo* assembly feature with default parameter values. The resulting assembly was converted to a blastable database using NCBI's makeblastdb tool. Using the *Asaphidion yukonense* sequences from Maddison (2012) as query sequences, the specimen 4606 transcriptome was searched using nBLAST, yielding single contig sequences for 28S, 18S, and Topo. For CAD, three overlapping contigs were found; they were identical in the overlapping regions, and were merged into a single sequence. For COI, three contigs were found, one containing full-length COI; the other two short fragments could be eliminated from consideration because they BLASTed to non-bembidiines in GenBank or contained stop codons.

Sequences have been deposited in GenBank with accession numbers KX907141 through KX907199 (Table 2). All sequences fall into the "genseq-4" category (Chakrabarty et al. 2013).

Alignment and phylogenetic analysis. The appropriate alignment was obvious for COI, CAD, and Topo,

as there were no insertion or deletions (indels) evident in the sampled sequences. The two ribosomal genes were aligned by MAFFT version 7.130b (Katoh and Standley 2013), using the L-INS-i search option and otherwise default parameter values. No data were excluded from analyses, other than trimming of ends of sequences that were notably longer than others.

Models of nucleotide evolution where chosen with the aid of jModelTest version 2.1.7 (Darriba et al. 2012; Guindon and Gascuel 2003). Among the models supported by GARLI, the models chosen by the Bayesian Information Criterion were 28S: TVMef+G, 18S: SYM+I+G, COI: TIM1+I+G, CAD: TVMef+I+G, and Topo: TrN+G. Likelihood analyses of nucleotide data were conducted using GARLI version 2.0 (Zwickl 2006), as orchestrated by the Zephyr package of Mesquite (Maddison and Maddison 2015a). Twenty-five search replicates were employed for maximum likelihood trees, and 200 replicates for bootstrap analyses.

Results

There are two distinct clades in each of the five gene trees (Fig. 5): one clade contains all of the larger, paler specimens, and another contains all of the smaller, darker specimens. As outlined below in the taxonomic section, the valid name for the larger, paler specimens is *Ocys harpaloides* (Audinet-Serville), and the valid name for the smaller, darker specimens is *Ocys tachysoides* (Antoine). Based upon the arguments presented below in the Discussion, we consider these different species, and for simplicity's sake we will speak of them as different species in this section, and use their valid names. Likelihood bootstrap support for these two species is strong (Table 3), being above 90% in at least two of the well-sampled genes.

In COI, there are 11 sites at which three to six of the *O. tachysoides* (all specimens except 2899) show a secondary peak in chromatograms; there are three different sites at which 1 to 3 *O. harpaloides* (2759, 2760, 2853) have a secondary peak. The secondary peaks suggest an alternative version of COI, which is presumably a nuclear copy, or "numt"(Thalmann et al. 2004), thus causing some uncertainty about the source of our sequenced COI. However, in spite of the uncertainty, the gene tree clearly supports two distinct species (Fig. 5).

A detailed examination of differences between and within each of these two species also reveals many sites at which they differ (Table 4). There are six fixed amino-acid differences between the two species across the three protein-coding genes.

Table 3. Support for the monophyly of each of the two species in data for each gene. Numbers shown are maximum likelihood bootstrap percentages.

Clade	28S	18S	COI	CAD	Topo
O. harpaloides	100	100	100	95	95
O. tachysoides	100	100	84	63	100

Table 4. Variation observed in the DNA sequences between and within species. The number of specimens examined per species ranged from 3 to 5, with each species represented by 2 to 3 localities. "nuc": nucleotides; "aa": amino acids. The * indicates that one of the fixed differences is an extra base in *O. tachysoides* relative to *O. harpaloides*.

	fixed differences between species		sites varying within species		% nuc differences between species
gene	nuc	aa	nuc	aa	
28S	22*	-	1	-	2.1-2.2
18S	15	-	1	-	0.9
COI	50	2	9	0	7.2-8.4
CAD	5	1	6	0	0.50-1.0
Topo	16	3	0	0	2.2

Table 5. Morphological features and habitats of the sequenced specimens. "Disc colour" refers to the colour of the elytral disc relative to the apex and lateral margins. Male genitalic type A is that shown in Fig. 6A, B, and genitalic type C is that shown in Fig. 5C, D; FA refers to female genitalia type shown in Figs 8A and 9A.

	Region	Disc colour	Genitalic type	Habitat
O. harpaloides	Northern Ireland	paler	A	ocean shore
O. harpaloides	Northern Ireland	paler	A	ocean shore
O. harpaloides	Republic of Ireland	paler	A	ocean shore
O. harpaloides	Belgium	slightly paler	FA	broadleaf forest
O. harpaloides	Belgium	slightly paler	A	coastal salt marsh
O. harpaloides	Belgium	± dark	A	broadleaf forest
O. harpaloides	Spain	paler	A	open woodland
O. tachysoides	Northern Ireland	dark	C	woodland
O. tachysoides	Northern Ireland	dark	C	woodland
O. tachysoides	Northern Ireland	dark	C	woodland
O. tachysoides	Northern Ireland	dark	C	woodland
O. tachysoides	Northern Ireland	dark	C	woodland
O. tachysoides	Belgium	dark	C	freshwater marsh
O. tachysoides	Belgium	dark	C	freshwater marsh
O. tachysoides	Germany	dark	C	woodland

Morphological characteristics were in general correlated with DNA sequences. All five males in the *Ocys harpaloides* clade in Fig. 5 had genitalia matching those shown in Fig. 6A, B, and all seven males in the *Ocys tachysoides* clade had genitalia matching Figs 6C, D (Table 5). Irish populations also had the colour of the elytral disc correlated with the gene tree clades, but the correlation was not as clear in Belgium (Table 5).

On the island of Ireland, all three *O. harpaloides* clade males were found on the ocean shore, at two localities, whereas all five *O. tachysoides* clade males were found in woodland habitats, at three localities (Table 5). That distinction was not as evident on the mainland of Europe, as two of the *O. harpaloides* specimens from Belgium were found in a broadleaf forest, inland (Table 5).

Discussion

Our data show that specimens in northern Europe referred to "*Ocys harpaloides*" belong to two species. The DNA data alone strongly suggests that two species live in Northern Ireland and Belgium: the gene trees for two certainly unlinked genes (nuclear 28S and mitochondrial COI) show the same clades for specimens that broadly overlap in geographic range, with specimens of both forms sampled from each region. This pattern, combined with identical patterns for 18S, CAD, and Topo, indicates that the two forms are not currently exchanging genes. This is confirmed by the correlated morphological traits, in particular the distinctive male genitalia.

Toribio (2013) recognized these two species in populations in southern Spain and Morocco, but did not recognize that *O. tachysoides* was as widespread as *O. harpaloides*.

By comparing our sequences with those presented by Raupach et al. (2016), we have confirmed that the two COI forms they documented correspond to *O. harpaloides* (their French specimens) and *O. tachysoides* (their German specimens).

Taxonomic section

The following key allows one to identify *Ocys* specimens from much of Europe, outside of those regions containing localized endemics (that is, outside of higher elevations in mountains in Spain, northern Africa, or Italy (Magrini and Degiovanni 2009; Neri et al. 2011; Netolitzky 1942), on islands (Magrini et al. 1998).

1 Pronotum with obtuse and rounded hind angles, with the hind margin protruding posteriorly in the middle relative to the hind angles; colour dark brown with bluish or greenish reflections ..... *Ocys quinquestriatus* (Gyllenhal)

– Pronotum with sharp hind angles, approximately a right angle, with the hind margin more or less straight; colour without notable metallic reflections 2

2 Elytra rufous or brown, in most specimens paler centrally with darker apical and lateral margins. Microsculpture of elytra consisting of more closely spaced, transverse lines with less of a tendency to form meshes (Fig. 7A, B). Aedeagus with ventral margin in most specimens bent downward toward the thinner apex; anterior sclerites of the internal sac more rounded; with a brush sclerite of normal size (Fig. 6A, B). Spermatheca more curved (Fig. 9A, B) .... *Ocys harpaloides* (Audinet-Serville)

– Elytra dark brown to black in colour, in some specimens paler along the suture, but entire discal region not paler than margins. Microsculpture of elytra consisting of less closely spaced, transverse lines with more of a tendency to form meshes (Fig. 7C, D). Aedeagus with ventral margin straighter, and apex wider, with a blunter tip; anterior sclerites of the internal sac more angulate; with a very small brush sclerite (Fig. 6C, D). Spermatheca straighter (Fig. 9C, D) .... *Ocys tachysoides* (Antoine)

Ocys harpaloides (Audinet-Serville, 1821)

Figs 2A; 3A; 6A, B; 7A, B; 8A; 9A, B; 10A, B; 11A, B

Bembidion harpaloides Audinet-Serville, 1821:78. Lectotype male, here designated, in the Dufour collection of the MNHN, examined (see Nomenclatural notes, below), with two labels: "5244" [handwritten], "LECTOTYPE Bembidion harpaloides Audinet-Serville 1821 designated 2016 D.R. Maddison and R. Anderson" [partly handwritten, with red lines around the border]. Type locality: Clavados, Bretagne, France.

Tachis rufescens Guérin-Méneville, 1823:123. Type specimens lost (see below). Type locality as specified in the original description: an island on the Seine River (presumably Île Seguin or Île Saint-Germain), Meudon, France. Neotype male, here designated, in the MNHN, labeled "JUVISY Aval RD 4-VI-45 M. DEWAILLY" [partly handwritten], "Ocys harpaloides Serv. M. Dewailly det." [partly handwritten], "NEOTYPE Tachis rufescens Guérin-Méneville designated 2016 D.R. Maddison & R. Anderson" [partly handwritten]. This specimen is from Juvisy-sur-Orge, Essonne, France, which is 18km SE of Meudon; it was formerly housed in the collection of Mr. Dominique Echaroux.

Ocys melanocephalus Stephens, 1828:10. Lectotype male, here designated, in BMNH, examined, with six labels: "BRITISH ISLES J. Stephens Coll. BM 1853- 46", "O melanocephalum" [handwritten], "♂", "harpaloides det Netolitzky" [handwritten], "NHMUK010363535" [with matrix barcode], "LECTOTYPE Ocys melanocephalus Stephens designated 2016 D.R. Maddison and R. Anderson" [partly handwritten, with red lines around the border]. Type locality: British Isles.

Bembidium dubium Wollaston, 1857:23. Holotype female, in BMNH, examined. Type locality: Madeira. Junior primary homonym of *Bembidium dubium* Heer, 1838. Synonymy tentative, based primarily on the colour of the type.

Nomenclatural notes. Interpretation of Audinet-Serville's name rests in part on discovery of his specimens, as his description is not sufficient to distinguish between the two species. Audinet-Serville notes that his specimen or specimens of *Bembidion harpaloides* are from "M. de Brébisson", presumably the botanist Louis Alphonse de Brébisson. Audinet-Serville's Coleoptera specimens were acquired by Léon Jean Marie Dufour (Thierry Deuve, pers. comm.), whose collection was incorporated into the

Figure 2. Live *Ocys*. **A**: *Ocys harpaloides* (Benderg Bay, Co. Down, Northern Ireland); **B**: *Ocys tachysoides* (Barnett's Demesne, Belfast, Northern Ireland).

Muséum National d'Histoire Naturelle in Paris. In March 2016, David H. Kavanaugh searched for several hours for the type series of *Bembidion harpaloides* Audinet-Serville in Dufour's collection at the MNHN, with help from Azadeh Taghavian. The following notes are in part from Kavanaugh (pers. comm.). Dufour's collection is well-organized, and in the section containing bembidiines, there is only one box with *Ocys*. It contains four pins with *Ocys* specimens. These are housed under the heading "*Bembidion (Tachys) rufescens*", a name in use around that time for this complex (e.g., Dejean 1831). In that region of the box, there are no additional pin holes, suggesting that there have been no specimens of *Ocys* removed from Dufour's collection. The four pins contain a total of eight specimens: the first two pins contain one specimen each, the third pin houses five specimens, each on a separate card, and the last pin houses a single specimen. The last specimen can be excluded from consideration, as its label contains a locality ("Carcassonne", in southern France) that differs from the locality stated by Audinet-Serville. The other three pins each have one or two labels: the first specimen has a label that apparently contains the number 249; the second the number 5244, and the remaining pin has two labels, one with a small amount of indecipherable text, the other with the number 124. All of the *Ocys* in Dufour's collection belong to this species, as determined by colour pattern and microsculpture. In addition, the first two are males, and dissection revealed genitalia that match genitalic type A (i.e., as in Fig. 6A, B). The labels on the three pins are in different handwriting, and the pins themselves differ in structure. Thus, it appears as if the three came from different sources, and likely only one of them represents authentic Audinet-Serville material. According to Antoine Mantilleri (Thierry Deuve, pers.

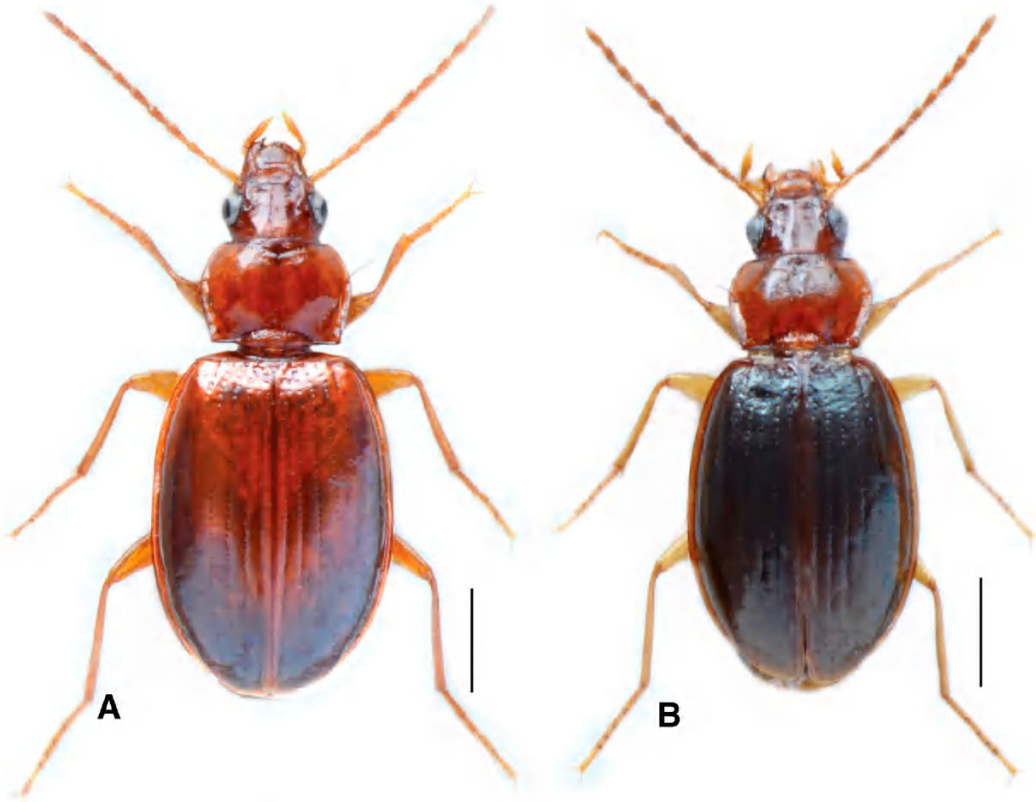

Figure 3. Habitus. **A**: *Ocys harpaloides* (Rinagree Point, Co. Londonderry, Northern Ireland); **B**: *Ocys tachysoides* (Annadale House, Belfast, Northern Ireland). Scale bars indicate 1 mm.

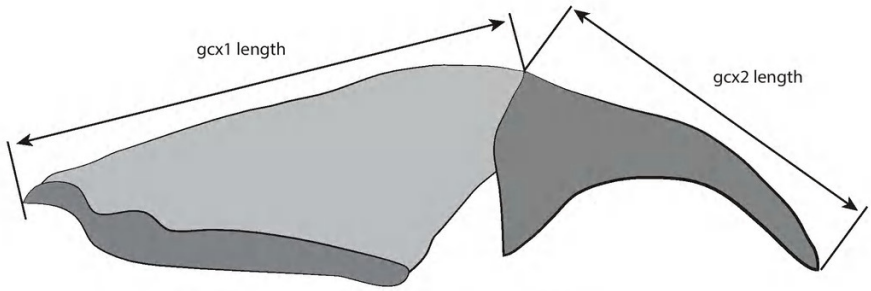

Figure 4. Diagram of gonocoxite of a female *Ocys* showing measurements taken.

comm.), the handwriting on the first specimen is likely that of Dufour. This suggests that the first specimen is not an authentic Audinet-Serville specimen, but rather an original Dufour specimen; however, it is possible that Dufour added a label to the specimen after having acquired Audinet-Serville's collection. The writer of the other labels is not known. We could discover no information about Audinet-Serville's handwriting, other than a single example of his signature, and thus we do not know if any of the labels might have been written by him, or whether they may have been added by later researchers. Thus, it is not clear which specimen on the three candidate pins is Audinet-Serville's, and it likely will never be determined with certainty. We consider the value in stabilizing the name more important than the uncertainty of the choice. As all of these specimens belong to the same species, as the specimen on the second pin is a male in sufficient condition and with undamaged genitalia, and as there is hint that the specimen on the first pin is not an Audinet-Serville specimen, we have chosen the second specimen as the lectotype of *Bembidion harpaloides* Audinet-Serville.

The collection of Félix Édouard Guérin-Méneville was incorporated into the Chaudoir collection at the MNHN (Cambefort 2006) in the Chaudoir magasin. David Kavanaugh and Azadeh Taghavian searched for types of *Tachis rufescens* Guérin-Méneville in the Chaudoir magasin. They found numerous *Ocys*, but every one could be eliminated as Guérin-Méneville specimens through consideration of the labels, as all specimens had a mechanically printed label (against other Guérin-Méneville specimens), and the labels specified a locality, collector, or collection that did not coincide

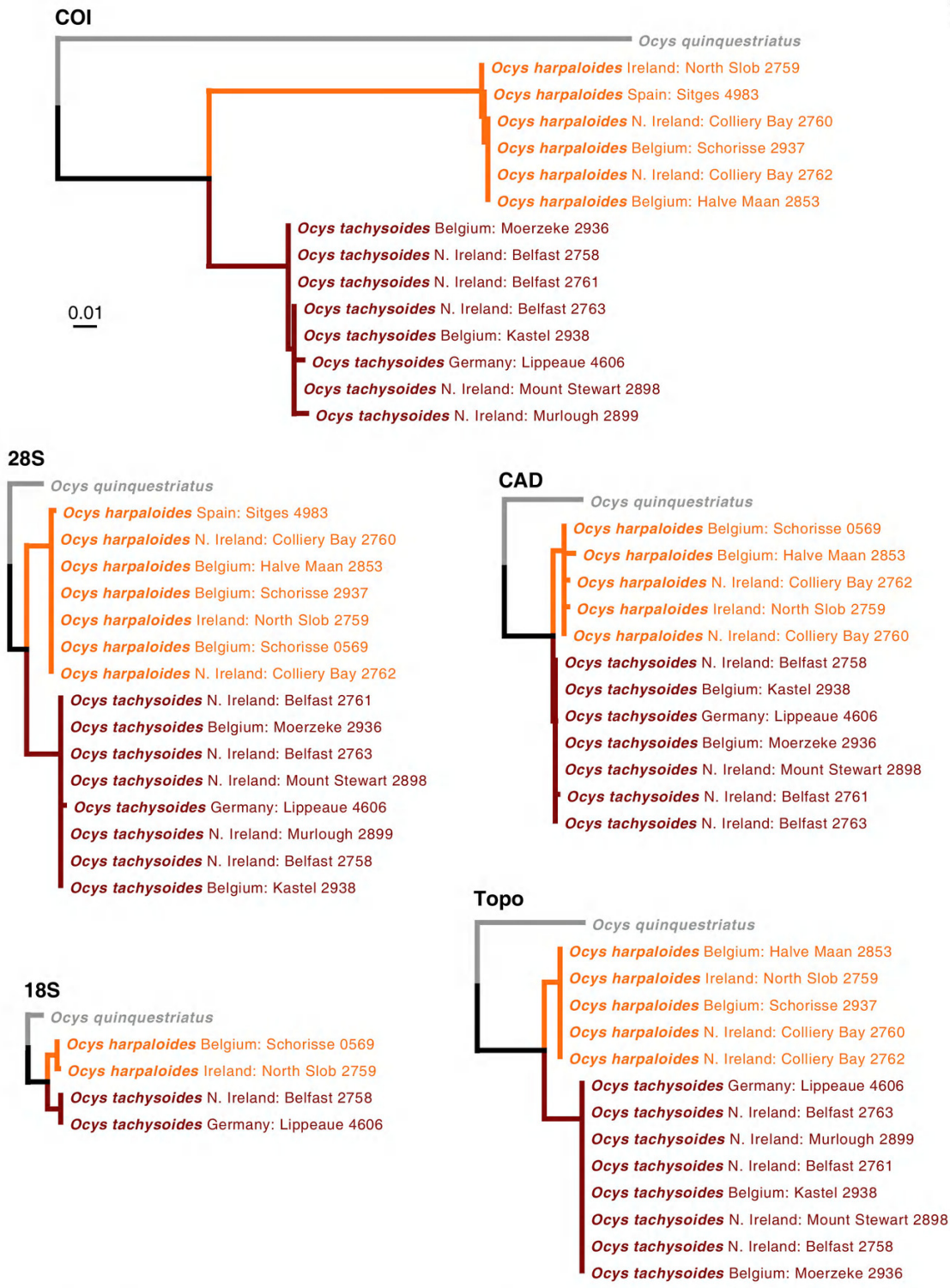

Figure 5. Phylogenetic trees of the five studied genes. Outgroups not shown. Branch length is shown proportional to relative divergence, as estimated by GARLI. The larger, paler specimens with genitalia as in Fig. 6A and B are shown in orange, and the smaller, darker specimens with genitalia as in Fig. 6C and D are shown in brown.

with those of the *Ocys* examined by Guérin-Méneville (Kavanaugh, pers. comm.). The Guérin-Méneville specimens of *Ocys* are thus lost. In the interests of stabilizing the nomenclature, we sought a neotype for *Tachis rufescens*. Within the MNHN, there are specimens from three localities around Paris (Vaires, Chelles, and Chalifert). All of these specimens belong to the following species, *Ocys tachysoides* (Thierry Deuve, pers. comm.); we have confirmed this for the specimens from Vaires by examination of male genitalia. However, the

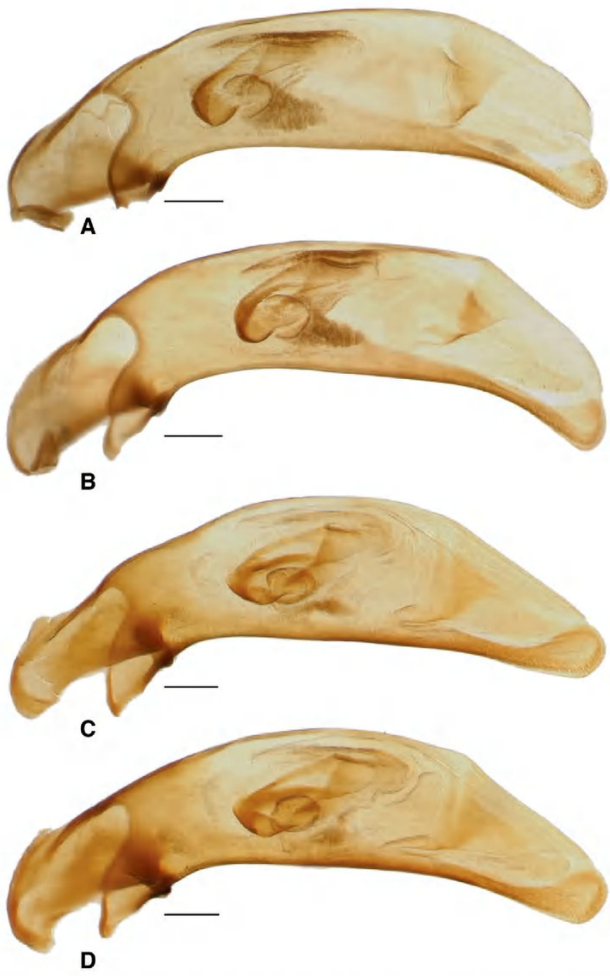

Figure 6. Male aedeagi. **A, B**: *Ocys harpaloides*, both from Colliery Bay, Ballycastle, Northern Ireland (**A**: voucher V100671, **B**: voucher V100670); **C, D**: *Ocys tachysoides*, both from Belvoir Forest, Belfast, Northern Ireland (C: voucher DNA2761; **D**: voucher DNA2758). Scale bars 0.1 mm.

lack of *Ocys harpaloides* in the twentieth-century specimens in the MNHN does not necessarily mean that the species was absent from Paris in 1823: in the 123 years that passed between the description of *Tachis rufescens* and the collection of the Viares specimens in 1946, the habitats around Paris may have changed. The possibility that *Ocys harpaloides* lived in Paris in the 1820s is made more likely by the presence, in the collection of Mr. Dominique Echaroux, of two series of specimens of *Ocys harpaloides* from just outside of Paris: one specimen from Bouray-sur-Juine and seven from Juvisy-sur-Orge. The latter is only 18km SE of the type locality of *Tachis rufescens*. Thus, both species have lived in the general Paris area, and a specimen of either species could be designated as a neotype. However, designation of the Vaires specimen, for example, would lead to *Ocys rufescens* as the name of the following species, with *O. tachysoides* as a junior synonym. This would change the name used for the following species in the Iberian Peninsula and Africa, where it is known as *O. tachysoides* (per Toribio 2013). In the interests of stability, we choose as neotype of *Tachis rufescens* one of the Juvisy-sur-Orge males; with this designation, *Tachis rufescens* is maintained as synonym of *O. harpaloides*.

In the BMNH, under "*melanocephalum*", are seven specimens (four males, three females) in the J. Stephens collection, all with the label "British Isles J. Stephens Coll. BM. 1853 – 46". We consider this to be the type series of *Ocys melanocephalus* Stephens. Three of the males in this series were dissected, and genitalia examined; they all match genitalic type A (i.e., as in Fig. 6A, D). We have chosen one of these specimens as the lectotype.

In the Wollaston collection of the BMNH, in Insecta Maderensia, Drawer 2, is a single teneral female, without a determination label. This specimen is presumed to be the holotype of *Bembidium dubium* Wollaston, 1854. Its colour suggests that it belongs to this species. However, the species membership of the type is of no nomenclatural importance, as the name is a junior primary homonym of *Bembidium dubium* Heer, 1838, itself a junior synonym of *Bemidion assimile* (Gyllenhal, 1810).

An additional name (*Carabus tempestivus* Panzer, 1799) is listed as a synonym of *O. harpaloides* by some authors (e.g. Stephens, 1828). However, this name is a synonym of *Trechus quadristriatus* (Schrank, 1781), as documented by Erichson (1837).

Diagnosis. Body length 4.2–6.1 mm (Toribio 2013 and our observations; average length of males 5.0 mm (n=5), of females 5.51 mm (n=5)). Head and pronotum a clear orange-brown; anterior and central part of elytra of the same colour, but sides and posterior region of elytra dark brown (Fig. 3), and with the epipleural gutter and suture yellow-brown. Microsculpture of elytral disc more transverse, with more close-set parallel lines and fewer meshes (Fig. 7A, B); density of microsculpture lines 25–26 per 0.1 mm (n=4 males). Hind margin of pronotum in most specimens straighter than in *O. tachysoides*, such that the hind margin is directed more or less laterally near the hind angles (Fig. 10A), occasionally with a slight emargination laterally (Fig. 10B). Elytra more parallel-sided, greatest width behind middle. Basal margin at shoulder slightly arcuate with a forward-directed concavity (Fig. 11A, B). Elytral striae 2 through 4 less marked in the apical third. Aedeagus with ventral margin bend slightly downward toward apex (Fig. 6A, B); apex more rounded. Anterior edge of central sclerite complex of internal sac more rounded; brush sclerite larger; dorsal membranes of internal sac darker. Gonocoxite relatively long (Fig. 8A), GCR=0.64–0.69 (average 0.67, n=5); spermathecal margin (opposite the efferent duct of the spermathecal gland) curved (Fig. 8A, B; n=5), with tip pointed toward duct of gland.

Geographic distribution (Fig. 12). In Africa, from Morocco, Algeria, and Tunisia. In Europe from Spain, France, Belgium, Italy, Ireland, and the United Kingdom. Examination of specimens in additional collections will likely show it to be more widely distributed.

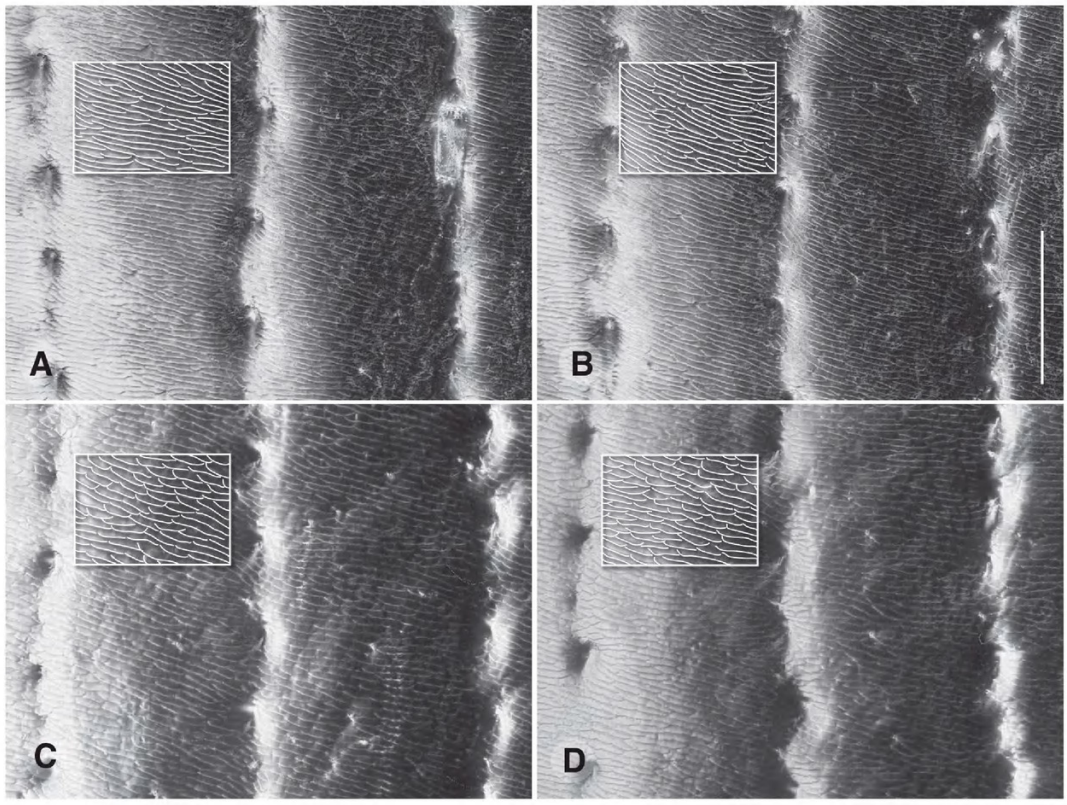

Figure 7. Microsculpture at center of disc of left elytron, third and fourth intervals. **A, B**, *O. harpaloides,* both from Colliery Bay, Ballycastle, Northern Ireland (A: voucher V100670, B: voucher V100672). **C, D**. *O. tachysoides* (C: Murlough NNR, Co. Down, Northern Ireland, voucher DNA2899, **D**: Mount Stewart, Co. Down, Northern Ireland, voucher DNA2898). Scale bar 0.1 mm.

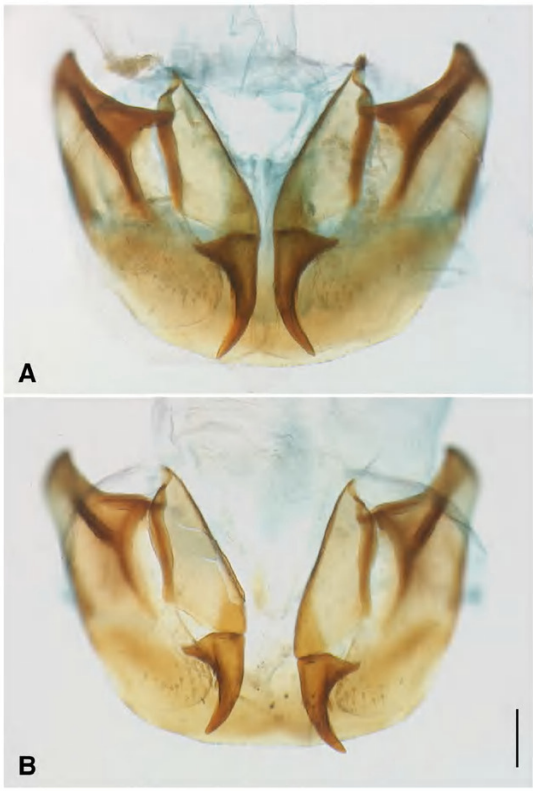

Figure 8. Female genitalia. **A**: *O. harpaloides*, Bos Ter Rijst, Schorisse, Belgium, voucher DNA0569. **B**: *O. tachysoides*, Murlough NNR, Co. Down, Northern Ireland, voucher V100983. Scale bar 0.1 mm.

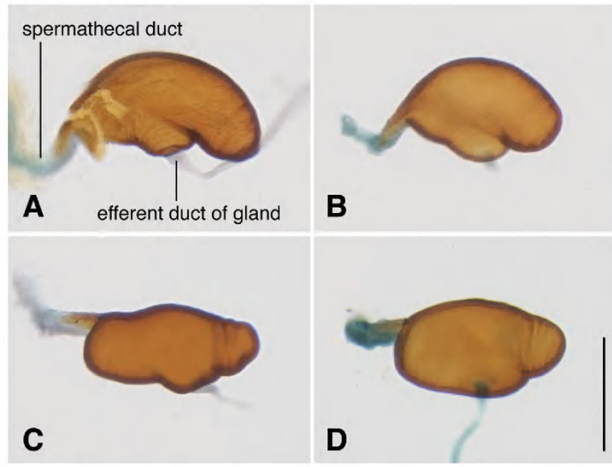

Figure 9. Spermatheca. **A, B**, *O. harpaloides* (**A**: Bos Ter Rijst, Schorisse, Belgium, voucher DNA0569, **B**: Beuda, Girona, Spain, voucher V100984) **C, D**. *O. tachysoides* (**B**: Murlough NNR, Co. Down, Northern Ireland, voucher V100983, **D**: Mount Stewart, Co. Down, Northern Ireland, voucher V100982). A bubble of air has been digitally removed from within the spermatheca shown in C. Scale bar 0.1 mm.

Specimens examined. In addition to type specimens, and those listed in Table 1, we examined specimens from Morocco: Tétouan (BMNH); Algeria (BMNH); France: Carcassone (MNHN), Bouray-sur-Juine (DE); Spain: San Roque (BMNH), Beuda, Girona (OSAC), and Sant Carles de Peralta (RAC).

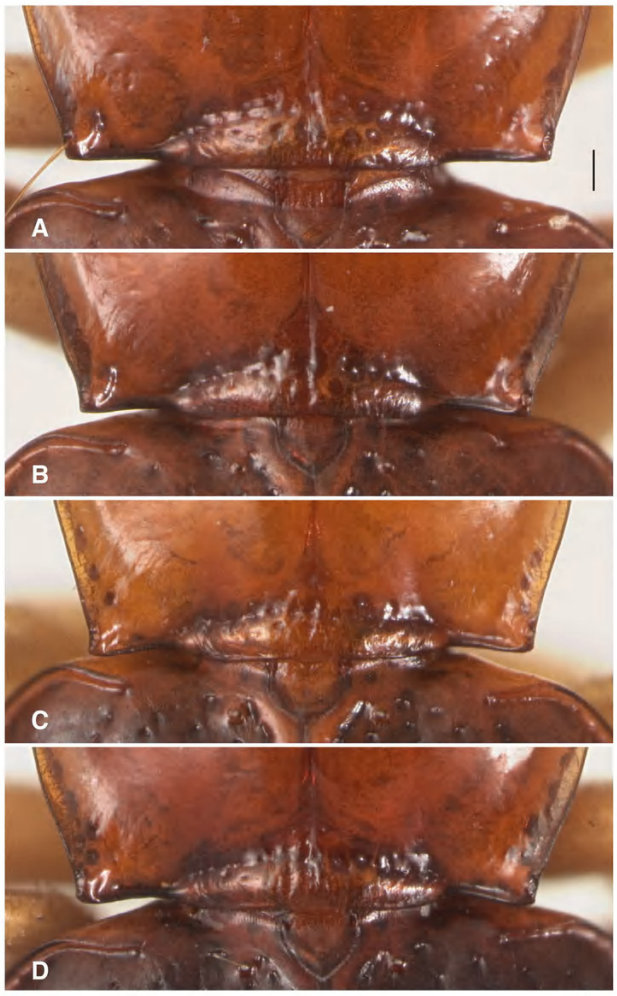

Figure 10. Hind margin of pronotum. **A, B**, *O. harpaloides* (**A**: North Slob, Wexford, Ireland, voucher DNA2759, **B**: Halve Maan, Oostende, Belgium, voucher DNA2853). **C, D**. *O. tachysoides* (**B**: Belvoir Forest, Belfast, Northern Ireland, voucher DNA2763, **D**: Murlough NNR, Co. Down, Northern Ireland, voucher DNA2899). Scale bar 0.1 mm.

Ocys tachysoides (Antoine, 1933)

Figs 2B; 3B; 6C, D; 7C, D; 8B, 9C, D; 10C, D; 11C, D

Bembidium harpaloides v. *tachysoides* Antoine 1933:79. Holotype female, in the MNHN, examined, with three labels: "O. Nefifik (Maroc) Antoine I.1928" [partly handwritten], "Holotype" [handwritten on red paper], "tachysoides Antoine det. m." [partly handwritten]. Type locality: Mouth of the Oued Nefifikh, east of Casablanca, Morocco (in the vicinity of 33.72°N, 7.34°W).

Nomenclatural notes. The type of *Bembidium harpaloides* v. *tachysoides* Antoine belongs to this species. We have examined external features of the holotype, including elytral microsculpture (which has a density of 21 lines per 0.1 mm), as well as shape of the spermatheca. Both second gonocoxites are broken in the holotype, and thus we were not able to measure their lengths.

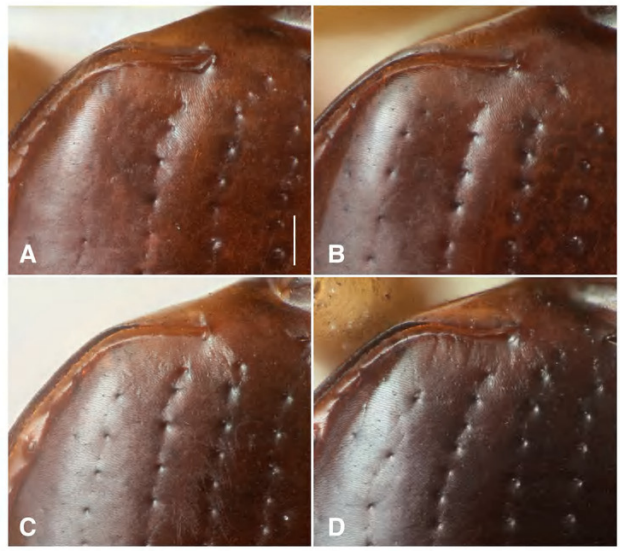

Figure 11. Left shoulder. **A, B**, *O. harpaloides* (**A**: North Slob, Wexford, Ireland, voucher DNA2759, **B**: Bos Ter Rijst, Schorisse, Belgium, voucher DNA2937) **C, D**. *O. tachysoides* (B: River Schelde, De Kramp, Moerzeke, Belgium, voucher DNA2936, **D**: Murlough NNR, Co. Down, Northern Ireland, voucher DNA2899). Scale bar 0.1 mm.

Diagnosis. Body length 4.0–5.8 mm (Toribio 2013 and our observations; average length of males 4.79 mm (n=7) of females 5.16 mm (n=7)). Head and pronotum a deep red-brown contrasting with the dark brown to blackish elytra (Fig. 3), only the epipleural gutters and sometimes sutures paler, light reddish-brown. Occasionally with a bluish lustre. Microsculpture of elytral disc less transverse, with more of tendency to form sculpticells (Fig. 7C, D); density of microsculpture lines 20–21 per 0.1 mm (n=4 males). Hind margin of prothorax with a slight emargination laterally, such that the hind margin at the hind angles is directed posteriorly (Fig. 10C, D). Elytra less parallel-sided, greatest width around middle. Basal margin at shoulder in most specimens more or less straight (Fig. 11C), relatively few specimens slightly arcuate with a forward-directed concavity (Fig. 11D). Elytral striae 2 through 4 more marked in the apical third. Aedeagus with ventral margin bend more or less straight (Fig. 6C, D); apex less rounded, blunt. Anterior edge of central sclerite complex of internal sac angulate; brush sclerite smaller; dorsal membranes of internal sac paler, less evident. Gonocoxite relatively short (Fig. 8B), GCR=0.55–0.60 (average 0.58, n=5); spermathecal margin opposite the efferent duct of the spermathecal gland more or less straight (Fig. 8C,D; n=5).

Geographic distribution (Fig. 12). In Africa, known from Morocco (Antoine 1955). In Europe, from Portugal (Sciaky 1998), Spain (Toribio 2013), France, Belgium, Germany, and the United Kingdom. Examination of specimens in additional collections will likely show it to be more widely distributed.

Specimens examined. In addition to the type specimen, and those listed in Table 1, we examined specimens from France: Vaires (MNHN).

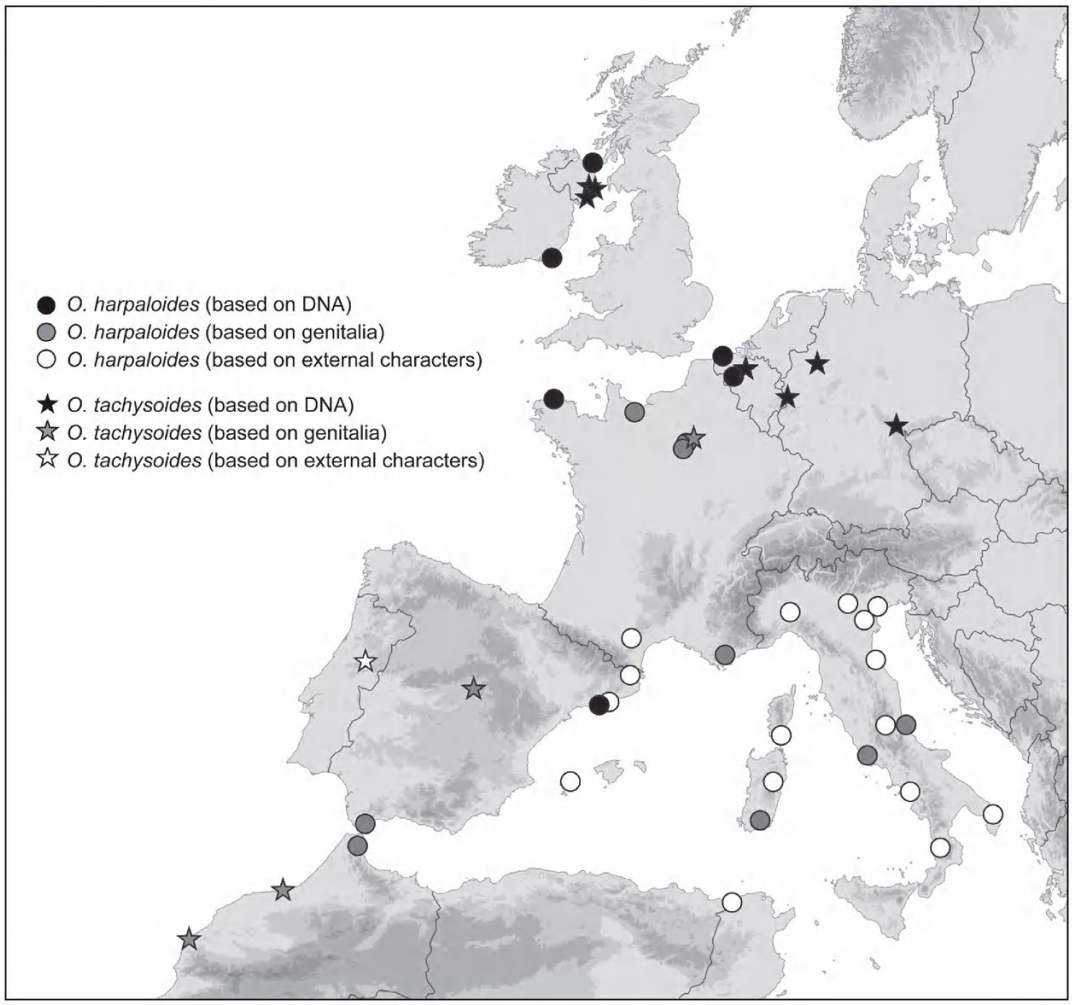

Figure 12. Distribution map of *Ocys harpaloides* and *O. tachysoides*. Only records of which we are confident are included. In addition to specimens we have examined, and those from Toribio (2013), Antoine (1956), and Sciaky (2009), we have included three publically available records from the Barcode of Life Database (http://www.boldsystems.org), corresponding to BOLD records GBCOU8213-14, GBCOU8667-14, and FBCOH116-12, as well as specimens identified by Luca Toledano and Paolo Bonavita in their collections (CTVR and PB, respectively) based upon images and diagnoses we provided to them.

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We are extremely thankful to the individuals who worked so hard on our behalf as we were attempting to locate original Audinet-Serville and Guérin-Méneville specimens at the Muséum National d'Histoire Naturelle, Paris, and to designate primary types for the associated names. Most importantly, Thierry Deuve patiently dealt with our many queries, examined numerous specimens, and visited Dominique Echaroux. His thoughtful responses, as we puzzled through the best approach to resolving the nomenclatural issues, were invaluable. Azadeh Taghavian's help in this regard can also not go unmentioned. In addition, David Kavanaugh spent many valuable hours while in Paris personally looking for *Ocys* types, and we could have not completed the project without his help; we are also thankful for his advice as we considered the best path, and for hand-carrying the Audinet-Serville material back from Paris. Several others aided these efforts as well, and we wish to thank them: Antoine Mantilleri (for his advice about Audinet-Serville material), and Paul Johnson (for providing copies of pages from Horn & Kahle). Finally, we wish to thank Mr. Dominique Echaroux, from Etréchy (Essonne, France), for providing specimens of *Ocys harpaloides* from his collection, and for allowing us to designate one of them as a neotype of *Tachis rufescens*.

We are also very thankful for Beulah Garner's stewardship of the specimens at the Natural History Museum in London, and for sending a portion of the type series of *Ocys melanocephalus* Stephens so that we might designate a lectotype.

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