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► **To cite this version:**

Olivier Denux, Emmanuelle Dauffy-Richard, Jean-Pierre Rossi, Sylvie Augustin. Rediscovery of the endangered species *Harpalus flavescens* (Coleoptera: Carabidae) in the Loire River. *Insect conservation and diversity*, 2017, 10 (6), 7 p. 10.1111/icad.12228 . hal-01605515

HAL Id: hal-01605515

<https://hal.science/hal-01605515>

Submitted on 25 May 2020

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SHORT COMMUNICATION

Rediscovery of the endangered species *Harpalus flavescens* (Coleoptera: Carabidae) in the Loire River

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Abstract. 1. The Loire River is one of the last European large rivers with important sediment dynamics and numerous sandbanks. The extraction of sediment from the riverbed during decades and the construction of levees for flood prevention have strongly affected and shaped the biodiversity of the Loire River.

2. Many species from pioneer riverbanks have been impacted with particular consequences for psammophilous insects. The ground beetle *Harpalus (Acardys-tus) flavescens* (Piller & Mitterpacher, 1783), is considered to have disappeared from the Middle Loire River for 40 years and is endangered everywhere in Europe.

3. In 2012 and 2013, we recorded two specimens of *H. flavescens* in Région Centre-Val de Loire (France), in the course of a survey dedicated to evaluating the impact of fluvial maintenance operations upon sediment and biodiversity dynamics.

4. The presence of *H. flavescens* may be linked to the interruption of riverbed extractions and the vegetation removal of sandbanks of the Loire River (ecosystem restoration).

Key words. Biodiversity, ground beetles, pioneer land, sand, shore, threatened species.

Introduction

Alluvial rivers have historically been an attractive source of sediment for economic development activities. But sediment extractions have produced many detrimental effects, including channel incision (sinking of riverbed), loss of riparian habitats and several other ecological and environmental impacts (Rinaldi *et al.*, 2005). With a length of 1012 km, the Loire River is the largest river in France and one of the last wild rivers of Europe (Bastien *et al.*, 2009). The path of the Loire and some of its tributaries often change due to an important morpho-sedimentary dynamics (Claude *et al.*, 2014; Rodrigues *et al.*, 2015). This leads to the transportation and natural accumulation of sediments and the formation of sedimentary bars (sandbars), which constitute one of the Loire River

characteristics. These formations have a peculiar dynamics due to the hydraulic characteristics (depth, speed) and shape of the channel (Wintenberger *et al.*, 2015a). Some of these bars are in stable position (forced bars) while others are very mobile in flood period (free bars). Free and forced bars can stabilise and support pioneer vegetation that can evolve to woody stages, where black poplar is prevalent (Wintenberger *et al.*, 2015b), or to more diversified woodland (island stage). Early stages of the succession correspond to pioneer sandy-gravel habitats and constitute a reservoir for a large number of species associated to sand. These species are referred to as 'arenicolous' (Torre-Bueno & Nichols, 1989; Hanson, 2007), 'sabulicole' (Hanson, 2007) or 'psammophilic/psammophilous' (Lewis, 1977; Thiele, 1977; Torre-Bueno & Nichols, 1989). Since the 1950s, the massive gravel extraction from the Loire riverbeds (e.g. 6.4 million tonnes in 1979: see Gasowski, 1994) has caused a significant imbalance between the amount of sediment extracted and the quantity naturally replenished by natural deposition

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(Claude, 2012). Coupled with containment, it has caused the incision of the Loire River and the lowering of the water line during low-water periods. These events have led to the disconnection of hydraulic attachments (backwaters, islands, sedimentary bars, etc.) and more generally to the decrease in the active bank of the river (Latapie *et al.*, 2014). In addition, the progressive cessation of agropastoralism and bank maintenances and the reduction in exceptional winter floods have led to the gradual plant colonisation of pioneer environments (Grivel, 2008).

At the same time, rivers and adjacent terrestrial habitats are impacted by eutrophication due to agricultural intensification and rising urban pressures (Smith *et al.*, 1999). This has led to the modification of the composition of the flora (Walker & Preston, 2006) and both herbivorous and predatory insects (Haddad *et al.*, 2000). In this context, the Loire River has been considered eutrophic since at least 1980 (Minaudo *et al.*, 2013).

Both these phenomena have contributed to the decline of pioneer sedimentary bars and to the modification of the species communities associated with these environments (e.g. loss of species richness, overgrowth of ordinary species, speeding up of the vegetation succession).

The ground beetle *Harpalus (Acardystus) flavescens* (Piller & Mitterpacher, 1783) (Fig. 1a) is an emblematic species of pioneer sand habitats such as continental aeolian sand dunes, river sandbanks, coastal dunes, or sandpits. It is a steno-xerophilous species (Luka *et al.*, 2009) that lives deep in the sand, especially among roots of various grasses, such as *Corynephorus*, *Psamma* and *Panicum*

(Lindroth, 1992). Adults appear and breed mostly in autumn. *H. flavescens* is a macropterous species (Hurka, 1996) but no flight observations have been made (Lindroth, 1992). Larvae live in sand, hibernate before the winter and complete their development during the next spring. Hence, sand exploitation has a direct impact on population dynamics particularly in riverbeds where extractions are conducted in autumn.

Due to its ecological specificities, *H. flavescens* is one of the endangered species that are strongly affected by the loss of sand areas and the degradation of its habitats, particularly in the rivers of Central and Eastern Europe (Bohác & Jahnova, 2015). Although *H. flavescens* used to be widely distributed in Europe (Homburg *et al.*, 2013), it has declined and has become very rare in some regions (Kugler *et al.*, 2008). Lindroth (1992) indicated that *H. flavescens* is very rare in Sweden, absent in Norway and Russian sector, and probably absent in Norway. In Slovak and Czech Republic, it is rare and localised (Hurka, 1996). In Denmark, *H. flavescens* has not been recorded since 1850 (Hansen & Jorum, 2014), in Belgium it is seriously threatened (Belgian Species List, 2015), in Switzerland and Germany it is an endangered species (Trautner *et al.*, 2005; Luka *et al.*, 2009), and it has been observed only in some northern parts of Italy (Allegro & Sciaky, 2001). In France, the species was historically present in the Tertiary sands of the Paris Basin, in the Loire and Allier basins (Jeannel, 1942; Bonadona, 1971; Velle, 2004), the Seille Valley, in the Bresse and Lyonnais (Sainte-Claire Deville, 1935). It has also been observed in

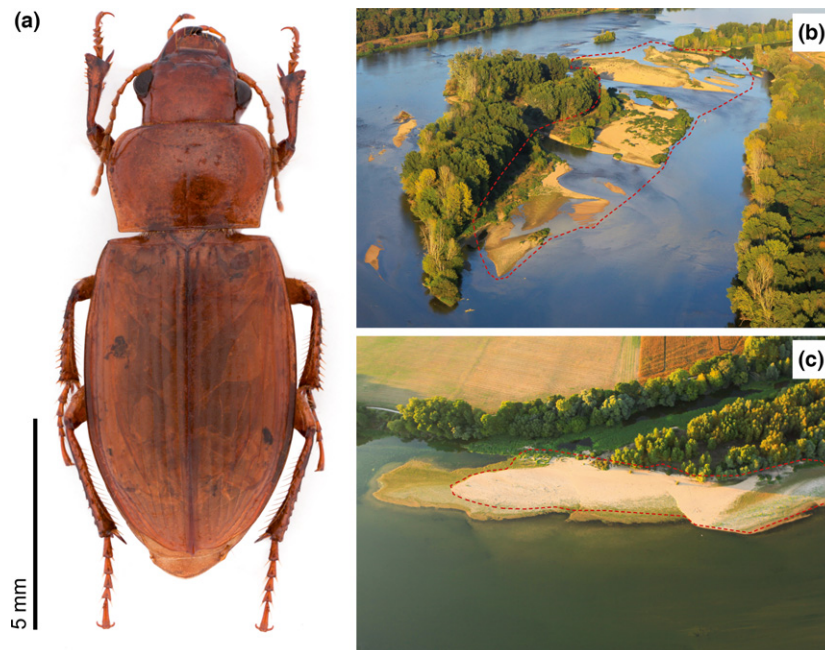


Fig. 1. (a) Habitus of *Harpalus flavescens* (copyright Olivier Denux); environment where *Harpalus flavescens* was found in Middle Loire River: (b) National Nature Reserve of Saint-Mesmin, Mareau-aux-Prés; (c), Baule. Red dotted line represents the favourable habitat areas of the species.

Alsace (Callot & Schott, 1993), Puy-de-Dôme and Pyrénées (Tronquet, 2014) and Picardie, Nord, Hautes-Pyrénées and Landes (Valembert, 1997). *H. flavescens* is declining (Coulon *et al.*, 2011) or endangered (Tronquet, 2014) in all areas where it exists. In the Loire Valley, *H. flavescens* has been considered as rare (Favarcq, 1876). In Loiret department, it was reported around Gien (Victor Pyot collection) and Orléans (Henry-Pierre Sainjon collection) until the early 20th century (Secchi *et al.*, 2009). Currently, *H. flavescens* is considered to be extinct in Région Centre-Val de Loire (Binon *et al.*, 2012).

The data reported in the present paper were collected in the course of a study of pioneer sediment dynamics and the associated biodiversity conducted in the Middle Loire between 2012 and 2015 (Villar, 2015; Wintenberger *et al.*, 2015a,b). Ground beetles constitute an ideal biological model to assess the dynamics of these habitats as the group is diversified with 1000 species in France (Coulon *et al.*, 2000) and its taxonomy well known (Coulon *et al.*, 2011). Ground beetles inhabit diversified habitats, their ecology is well documented (Thiele, 1977; Valembert, 1997; Desender *et al.*, 2010) and robust sampling methods are available (Work *et al.*, 2002). They are good biological indicators of soil or sediment, humidity, coverage, density and type of vegetation, trophic level (Rainio & Niemela, 2003; Paillet, 2007; Lamberts *et al.*, 2008, 2009) and their disruption (Avgn & Luff, 2010; Kotze *et al.*, 2011). Many ground beetles are capable of flying and can disperse widely (Thiele, 1977) and are thus adapted to rivers with natural dynamics. As such, ground beetles have been used in numerous biodiversity studies in forest (Magura *et al.*, 2001; Richard, 2004), in poplar plantations (Allegro & Sciaky, 2003; Denux *et al.*, 2007; Elek *et al.*, 2010), in agricultural areas (Liu *et al.*, 2010; Sonoda *et al.*, 2011; Holland *et al.*, 2012), and in alluvial areas (Lamberts *et al.*, 2008; Januschke *et al.*, 2011). In alluvial areas, sand and gravel sediment bars are important for ground beetles (Lachat *et al.*, 2001), where they are good bio-indicators for the management and restoration of river ecosystems (Gerisch *et al.*, 2006; Januschke *et al.*, 2011; Januschke & Verdonschot, 2016) and hydrological conditions (Gerisch *et al.*, 2006). Finally, they colonise all pioneer riparian habitats, including nude sediment bars where few organisms are usually present. A large sampling campaign was carried out in various habitats of the Région Centre-Val de Loire and offered a unique opportunity to re-examine the distribution of rare or extinct ground beetle species such as *Harpalus flavescens*.

Materials and methods

The study was conducted in France, in the Région Centre-Val de Loire on four sites of Loire River between Châteauneuf-sur-Loire (45), upstream, and Blois (41), downstream (see Fig. S1), with a more important sampling effort on the islands of National Nature Reserve of Saint-Mesmin (see Fig. S1, sector b [47°51'54.0"N,

001°46'56.3"E]). We studied other Loire River sections, located upstream in the areas of Sandillon and Saint-Denis-en-Val (see Fig. S1, a1 sector [47°51'30.4"N, 002°03'07.0"E] and A2 sector [47°53'14.5"N, 002°00'08.9"E]) and downstream in the areas of Baule, Suèvres and Saint-Dyé-sur-Loire (see Fig. S1, c sector [47°47'40.7"N, 001°40'52.7"E] and D sector [47°39'34.2"N, 001°28'58.5"E]).

Ground beetles species abundance was surveyed from 2012 to 2014 in five pioneer habitats of the Middle Loire River: sandy formations, gravelly formations, mudflats and cracked soil, grassland and poplar coppice.

Ground beetles were sampled using two methods. We used pitfall traps filled one-third full by 20% Mono-Propylene Glycol (also called Propanediol) because it is the most common technique to sample ground-moving species (Work *et al.*, 2002). Mono-Propylene Glycol, combined with saturation of salt and a wetting agent (to decrease the surface tension of the water and thus lead to the drowning of the insects) allows a very good conservation of the specimens between two sampling periods (2 weeks lag). It also limits the evaporation of the liquid in the pitfall trap (Denux, 2005) which is an important issue when sampling in xero-thermophilic habitats where sunshine and heat can dry out a pitfall trap in a few days.

Among riparian and mudflat ground beetles, very common along the Loire River, many species are generally difficult to catch in the pitfall trap (e.g. small species). Quadrat samples were therefore used to complete the pitfall traps and to optimise our sampling (Bigot & Gautier, 1981; Dajoz, 2002). The quadrat samples were used with a square metal quadrat (0.25 m²) applied to the soil to limit invertebrate escape (Andersen, 1995). Individuals were caught following three complementary actions: (i) visible individuals were immediately caught, if necessary using a mouth aspirator equipped with a recipient, (ii) The surface of the quadrat was watered to cause a phenomenon of escape of individuals, which were immediately captured, and (iii) Stones, gravel and debris were removed to extract hidden individuals.

Pitfall traps were used from early July to late September, between 2012 and 2014, and checked every 2 weeks. The sampling by quadrat was carried out when the climatic conditions (heat and sunshine) were favourable: on 31 July 2012, 08 August 2012, 14 July 2013, 02 September 2013, 15 September 2013 and 03 September 2014.

Results and discussion

During the study period, 754 pitfall trap samples and 223 quadrat samples were collected for a total of 99 species and 8743 individuals (pitfall traps: 97 species and 8055 individuals, quadrats: 29 species and 679 individuals). Among these specimens, two females of *H. flavescens* were collected with pitfall traps. No individuals of *H. flavescens* were captured with quadrats, in spite of the removing of stones, gravel and debris in the upper soil layer. In most

cases of active capture of *H. flavescens*, individuals were hidden below stones and debris.

The first individual was captured on 03 September 2012 on an island of the National Nature Reserve of Saint-Mesmin (Mareau-aux-Prés, 45; Fig. 1b). The pitfall trap was located on a dry sand/gravel substrate with little vegetation (about 10% herbaceous cover) and at 10 metres from the river (see Fig. S2a). In the pitfall trap, *H. flavescens* was found with two other Carabidae, *Amara fulva* (Müller, 1776) and *Lionychus quadrillum* (Duftschmid, 1812).

The second individual was caught on 20 August 2013 in Baule (45), on the right bank of the Loire (Fig. 1c), in cracked soil with silt/clay deposit, at 15 metres from the water, with about 40% grass cover (see Fig. S2b). It was found with the following species: *Bembidion quadrimaculatum* (Linnaeus, 1761), *Bembidion femoratum* Sturm, 1825, *Pterostichus anthracinus* (Illiger, 1798), *Oxypselaphus obscurus* (Herbst, 1784), *Chlaenius nigricornis* (Fabricius, 1787) and *Chlaenius tibialis* Dejean, 1826.

The relative abundance of *H. flavescens* was 0.025%, for all pitfall traps and habitats sampled in our study. Considering the ecological criteria that appear to be the most suitable to this species (Table 1) and filtering our pitfall trap sampling data on these criteria ($\geq 50\%$ sand; 'dry vegetation' $\leq 50\%$, sampling period between early August and late September), the relative abundance of *H. flavescens* in favourable pitfall trap samples was 0.4% (for 76 pitfall trap samples).

Harpalus flavescens had not been observed for at least 40 years in the Région Centre-Val de Loire and had been considered extinct (Binon *et al.*, 2012). Our study provides a proof of the recent presence of *H. flavescens* in the Middle Loire. Hence, it will be necessary to change the protected status of this species in the Région Centre-Val de Loire, from disappeared species to threatened species (Binon *et al.*, 2015). Several hypotheses may explain the rediscovery of this species in the Middle Loire.

(1) *H. flavescens* is a rare and autumnal species (Luka *et al.*, 2009), and inhabits habitats that are rarely studied by entomologists. This could explain the low probabilities of capture, without specific studies of this species. Our important sampling effort (754 pitfall trap samples and 223 samples per quadrat for a total of 8743 ground

beetles identified) may explain the rediscovery of this rare species erroneously considered to be extinct in the Région Centre-Val de Loire. Other studies, however, including beetles were carried out in the Middle Loire, particularly in the National Nature Reserve of Saint-Mesmin, where two surveys have been conducted: Pratz and Roger (1998) used active insect collection and Jaulin (2004) with 166 samples and several methods (traps and actively collect insects). In a legislative framework, a number of inventories were also conducted by entomologists in this National Nature Reserve: in the period 1997–2016, 482 species of beetles including 70 ground beetles species were identified (extraction from the National Nature Reserve of Saint-Mesmin database). In all of these cases, *H. flavescens* was not observed. This suggests that sampling power may not be the sole explanation behind the rediscovery of *H. flavescens* in the Middle Loire.

(2) The cessation of sand mining in the Loire Riverbed (Dambre, 1996) did not prevent the river incision in some locations (Latapie *et al.*, 2014), nor did it hamper the revegetation of the bed (Braud, 2012). As the current hydrology of the river does not curb this process, the State services have chosen to carry out mechanical interventions on the river since the 1990s, with levelling, scarification and devegetation actions. In Europe, the effects of hydromorphological river restoration were studied in 20 rivers, using a standardised monitoring and sampling design (Kail *et al.*, 2016; Muhar *et al.*, 2016). Januschke and Verdonschot (2016) indicated that river restoration had a significant positive effect corresponding to an increased richness of specialist riparian ground beetles. Another study showed that river re-braiding measures have increased the habitat diversity and the number of ground beetle species (Jähnig *et al.*, 2009). These management operations contributed to the restoration of pioneer habitats such as sandy habitats with little vegetation favouring the return of species such as *H. flavescens*. *H. flavescens* may thus have benefited from these restoration operations to gradually recolonise the Middle Loire River from refuge sites (e.g. head of river).

(3) Winter floods are able to carry plant debris and insects in winter state of torpor over substantial distances (Colas, 1974), which become stranded on the banks and islands of the Loire, far from their area of origin. Such dispersion was reported for several ground beetles (Paillet, 2007). In our case, this would correspond to the transportation of individuals of *H. flavescens* from the upstream of the Loire or Allier River to our sampling sites. Finally, although no active flight has ever been observed, *H. flavescens* has full wing development (Lindroth, 1992). Hence, it is not strictly speaking impossible that individuals could have flown from refuge areas to the Loire River.

The rediscovery of *H. flavescens* raises questions about the origin of individuals observed in the Middle Loire River (local population or individuals from refuge areas), the stability of the populations (possibility of full life cycle, population size) and the anthropogenic impacts

Table 1. Ecological preferences of *Harpalus flavescens*.

Ecological preferences	Bibliographic references
Psammophilous	Bonadona (1971), Hurka (1996), Jeannel (1942), Kugler <i>et al.</i> (2008), Thiele (1977), Valemberg (1997)
Thermophilous and/or xeric habitats	Kugler <i>et al.</i> (2008), Luka <i>et al.</i> (2009)
Bare substrate or little vegetation	Luka <i>et al.</i> (2009), Thiele (1977), Tietze (1973)
Autumnal species	J. Coulon (pers. comm.), Luka <i>et al.</i> (2009)

(end of sediment extractions and hydromorphological river restoration). New inventory based on intensive sampling targeting its specific habitats are necessary to confirm the presence of *H. flavescens* and better document its abundance and spatial distribution. The complete cessation of sand exploitation in the Loire riverbed in 1995, the restoration engineering of shores and islands and more generally, the integration of biodiversity in the riverbed management strategies contributed to the restoration of pioneer habitats, hence allowing the recolonisation of the river banks by rare or extinct species such as *H. flavescens*.

Acknowledgements

We thank Aminata NDhyae, Vanessa Imbault, Audrey Bras and Alexis Bernard for their technical support in the field. Many thanks to Michel Chantreau and Damien Hémeray, Conservator and technician of the National Nature Reserve of Saint-Mesmin, for entomological data and field support. We also thank Michel Binon, Jacques Coulon, Bernard Lemesle and René Pupier for *Harpalus flavescens* distribution data. We express our gratitude to Stéphane Rodrigues (UMR CITERES, University of Tours, France) for information and corrections on the morpho-sedimentary aspects of the Loire River in the manuscript. We thank the pilot of the ULM, Franck Robinet, who helped us to take the aerial photographs (Fig. 1b, c). This study was included in BioMareau project, coordinated by Marc Villar (INRA, UR588 AGPF, France). It was supported financially by the Région Centre-Val de Loire and European Union. European Union is engaged on the Loire Basin with the European regional development fund (ERDF).

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12228:

Fig. S1. Location of sampling sites: Sandillon (a₁), Saint-Denis-en-Val (a₂), Mareau-aux-Prés (b), Baule and Lailly-en-Val (c), Suèvres and Saint-Dyé-sur-Loire (d). Green points represent pitfall trap sampling and red stars represent quadrat sampling.

Fig. S2. Habitats where *Harpalus flavescens* was caught: (a) dry sand with sparse vegetation; (b) cracked soil with silt/clay deposit awaiting vegetation.

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Accepted 10 March 2017

Editor/associate editor: Raphael Didham