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New finds of the click beetle *Agriotes sordidus* (Illiger, 1807) and an overview on its current distribution in Germany

Neue Funde des Schnellkäfers *Agriotes sordidus* (Illiger, 1807) mit einem Überblick über seine aktuelle Verbreitung in Deutschland

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Abstract

Some of the most damaging wireworms in middle European arable land belong to the genus *Agriotes*. One of the five species of this genus which are of economic importance in Germany, is a newcomer. The species in question, *Agriotes sordidus*, is essentially western Mediterranean in its distribution, however, showing a range extension to the north in Western Europe in recent decades. In Germany *A. sordidus* now shows a continuous distribution along the Upper Rhine valley and its tributaries (e.g. Neckar, Nahe, Main), as was shown recently. The species is also recorded in the countrywide click beetle monitoring with additional finds at the southern edge of the Cologne lowland (first find in North Rhine-Westphalia), the region of Zusmarshausen in western Bavaria, and most surprising, two locations close to the North Sea coast in Lower Saxony and Schleswig-Holstein (first find in this federal state). The latter two sites and also the Bavarian site near Zusmarshausen are far from the area of continuous distribution in southwestern Germany. As northern German specimens differed visually from the southern German finds the morphological determination was verified by PCR. Therefore *A. sordidus* is already more widespread than previously thought, but still missing in most regions. No records exist from eastern Germany yet. However, the habitat preferences and the damage potential of this species are still not fully understood. The spread of *A. sordidus* should be monitored further.

Key words: Wireworms, click beetle monitoring, *Agriotes sordidus*, distribution, pheromone traps

Zusammenfassung

Die Schnellkäfergattung *Agriotes* beinhaltet einige der in Ackerbaukulturen schädlichsten Drahtwürmer. Von den fünf in Deutschland als wirtschaftlich bedeutend eingestuften *Agriotes*-Arten ist eine, *Agriotes sordidus*, als Neuankömmling mit rezent erfolgter Nordausbreitung zu betrachten. Diese ursprünglich westmediterrane, als sehr schädigend angesehene Art wird auch im bundesweiten Schnellkäfermonitoring erfasst. Sie hat inzwischen ein geschlossenes Gebiet im Oberrheingraben besiedelt, sich entlang einiger Rhein Nebenflüsse weiter ausgebreitet und ist über den Mittelrhein bis an den südlichsten Rand der Kölner Bucht vorgestoßen. Dieser Standort Westerhausen stellt wahrscheinlich den ersten Fund für Nordrhein-Westfalen dar. Ein weiterer Fund bei Zusmarshausen stellt den wohl ersten Fund für Bayern abseits des an den Oberrhein anknüpfenden Vorkommens entlang des Mains dar. Überraschend sind Funde in Küstennähe in Niedersachsen und Schleswig-Holstein weitab vom geschlossenen Verbreitungsgebiet im Südwesten Deutschlands. Norddeutsche Tiere unterschieden sich zwar optisch von den Tieren aus dem südlichen Verbreitungsgebiet, aber die morphologische Bestimmung wurde mittels PCR bestätigt. *A. sordidus* ist demnach in Deutsch-

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land weiter verbreitet als angenommen. In den meisten Gebieten fehlt er aber noch, so z.B. im Osten Deutschlands. Die Habitatpräferenzen sowie das Schadpotential in den neu besiedelten Gebieten sind damit aber nicht geklärt. Die Ausbreitung dieser Art sollte weiter überwacht werden.

Stichwörter: Drahtwürmer, Schnellkäfer-Monitoring, *Agriotes sordidus*, Verbreitung, Pheromonfallen

Introduction

The genus *Agriotes* is known as the most damaging genus of the family Elateridae in Europe. The larvae (wireworms) of this genus feed on subterranean plant parts (roots, rhizomes, tubers, bulbs) and can cause severe losses, while adults (click beetles) feed on pollen and green plant tissue without inflicting significant damage. *Agriotes* wireworms are extremely polyphagous. Plants attacked are potato, maize, cereals, beet, clover, several grasses, sunflower, strawberry, several vegetables, spices like parsley, onion and others, but also several weeds and wild plants.

In Germany, five *Agriotes* species are considered to be of economic importance: *Agriotes lineatus*, *Agriotes obscurus*, *Agriotes sputator*, *Agriotes sordidus*, and *Agriotes ustulatus*. As it can be quite difficult to distinguish between some of these species in their larval stage, wireworms have not always been identified to species in published work. Therefore, with the availability of sex pheromone traps for male click beetles of these five species the countrywide click beetle monitoring was initiated to learn more about their distribution on agricultural land in Germany.

Among these five species, *Agriotes sordidus* still has the most restricted distribution in Germany. It has been described essentially as a Western Mediterranean species, in the North reaching Lorraine in France, Belgium, and the Upper Rhine valley in Germany (LOHSE, 1979; JEUNIAUX, 1996; LAIBNER, 2000). Since a long time, this species has already been considered an important economic pest in Italy (e.g. RUSEK, 1972; BURGIO et al., 2012). In Spain it is also one of the most common elaterids (SÁNCHEZ-RUIZ et al., 1998). In France, LESEIGNEUR (1972) noted that *A. sordidus* was common in the south, but rare in the northern part of the country and seemed to be absent from the east (Alsace and Lorraine). Since then it has shown considerable northward spread. Nearly three decades later COCQUEMPOT et al. (1999) confirmed that it was the dominant species on agricultural land in the south (Aquitaine, Béarn, Languedoc, Lower Rhone valley). They observed its presence in the Mediterranean and on the Atlantic coast from the Pyrenees northwards to the Loire river but its absence in the Bretagne and the Normandy, while in the east it now reached the Alsace. In the United Kingdom it seems to be known to date only from a few scattered records mainly in the south (<http://elateridae.co.uk/species-accounts/agriotes-sordidus/>).

In the Netherlands the first record consists of two specimens of *Agriotes sordidus* (Illiger) that have been collected on a mudflat near Terneuzen, Zeeland, in April 2002 (VAN NUNEN, 2007).

In Germany the first known discovery of *Agriotes sordidus*, a single male, occurred in 1972 in the Kaiserstuhl area in the Upper Rhine valley (LOHSE, 1979; ZEISING, 1984). Both a northward spread and a considerable increase in numbers occurred over the last decades, making *A. sordidus* now a common species in the Upper Rhine valley (BURGHAUSE and SCHMITT, 2011). The most surprising recent record of this species came from a coastal area in Lower Saxony in 2006 (GOLLKOWSKI, 2007), far away from the Upper Rhine valley.

A. sordidus is considered to be the most harmful wireworm in southern regions where it is common. Its life cycle in the south is fairly short with 2–3 years (FURLAN, 2004) compared to 3–5 (–6) for Middle European species of economic importance like *A. lineatus*, *A. obscurus* and *A. sputator*. According to DEDRYVER et al. (2009) the fast development leads to higher damage, especially in potato. If the development of *A. sordidus* in Middle Europe is still faster than that of other species it may be more harmful. Additionally, it might be able to cope with drier conditions due to its origin. Therefore, a good knowledge of its biology, distribution and habitat preferences in Germany is important.

Material and Methods

Click beetle sampling

The countrywide click beetle monitoring was initiated to learn more about the *Agriotes* species distribution on agricultural land in Germany. The traps used were Yatlor traps from ROSA Micromecanica s.a.s. (San Donà di Piave, VE, Italy). The sex pheromone of *A. sordidus* (Geranyl hexanoate, TÓTH et al., 2002) and the other four species monitored came from Csalomon (registered trademark of the Plant Protection Institute, Centre for Agricultural Research, HAS, Budapest, Pf. 102, H-1525, Hungary). In Germany, *A. sordidus* traps were placed at 69 locations in 2011 and at 80 locations in 2012 (partly the same as in the previous year). All except one of the locations were part of the monitoring with five traps for five species at the site. The exception was a location near Sehestedt (Jadebusen, Lower Saxony) in 2012, where *A. sordidus* had been recorded previously (GOLLKOWSKI, 2007). At this site only a trap for *A. sordidus* was placed. Additionally to these sites some recent data from literature (BURGHAUSE and SCHMITT, 2011), the web (<http://www.kerbtier.de/cgi-bin/deFSearch.cgi?Fam=Elateridae>) and personal communication (H. SCHNELLER, J. SCHMITT) on the occurrence of *A. sordidus* were added to have the map as complete as possible. Traps were emptied at different intervals depending on the site, either weekly or in longer periods of 10 days to two weeks. All beetles caught were morphologically determined according to LOHSE (1979) and LAIBNER (2000). Beetles from the northern German

sites in Schleswig-Holstein and Lower Saxony were additionally determined by using PCR, as the Northern German beetles differed visually from the Southern German specimens.

PCR

DNA extraction. The DNA was extracted from the wireworms or their imagines using the CTAB method (DAY and SHATTOCK, 1997). DNA was also extracted from beetles which were stored in 70% or 80% alcohol, respectively.

PCR Performance. The PCR was conducted using a total of 10 µl volume and the Hybaid PCR Sprint Thermal Cycler (Thermo Electron GmbH, Dreieich, Germany), with the following conditions: 3 min. 96°C, 50 sec. 95°C, 45 sec 61°C, 50 sec 72°C for a total of 45 cycles and a complementation of the PCR-products at 72°C for 5 min. The PCR-products were loaded on a 1.4% agarose gel, separated via electrophoresis and photographed after an ethidium bromide staining. The primers for the different *Agriotes* isolates were applied according to the publication of STAUDACHER et al. (2011).

Results

The morphological determination of click beetles caught in pheromone traps is unavoidable, as mis-catches commonly occur. A catch in the pheromone traps for *A. sordidus* is not necessarily this species which may be the reason for erroneous reports of this species. Click beetles from the *A. sordidus* traps all over Germany turned out to belong to several different species (Fig. 1). The same problem applies to catches with the pheromones of other species, but it appears to be quite pronounced in *A. sordidus*, as the species does not yet occur at many sites. If catches with this trap were taken for *A. sordidus* uncritically, the result would be that the species occurred all over Germany. Other specimens caught in the *A. sordidus* traps mainly belonged to the species *A. gallicus*, a smaller, very flight-active species that is not known to be harmful

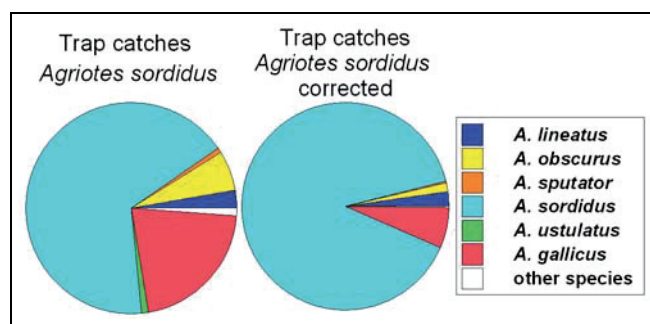


Fig. 1. Catches of the *Agriotes sordidus* trap over all sites in Germany 2011 (n = 2646 click beetles) and corrected catches only for sites where *A. sordidus* was known to occur (n = 1978 click beetles). All five species from the monitoring program occurred in the trap with the *A. sordidus* pheromone, plus *Agriotes gallicus* and some click beetles from other genera.

to crops. At sites where *A. obscurus* was common some beetles of this species were also attracted to the traps, but less than to the trap with their own pheromone. Looking only at the sites where *A. sordidus* really did occur (corrected catch) it is apparent that the pheromone still works quite well for this species.

Looking only at the corrected catch the mis-catches are much less pronounced with about one eighth compared to one third of the beetles for all sites.

The German wireworm and click beetle monitoring documents that the species *Agriotes sordidus* nowadays is common in the Upper Rhine valley and has spread from this area along some tributaries of the Rhine (Nahe, Main, Neckar). The region around Schweinfurt and Würzburg on the Main river is the most eastern outpost of continuous distribution (Fig. 2). The Wetterau region north of Frankfurt has also been colonized (finds at Münzenberg in 2011 but not in 2012). The species also has spread further north through the Middle Rhine valley, reaching now already southernmost North Rhine-Westphalia (Westerhausen-Bad Hennef at the geographic latitude of Bonn, two individuals in 2011, one in 2012). This seems to be the first documented occurrence in North Rhine-Westphalia.

But there are also some finds from isolated sites away from the area of continuous distribution. These sites are

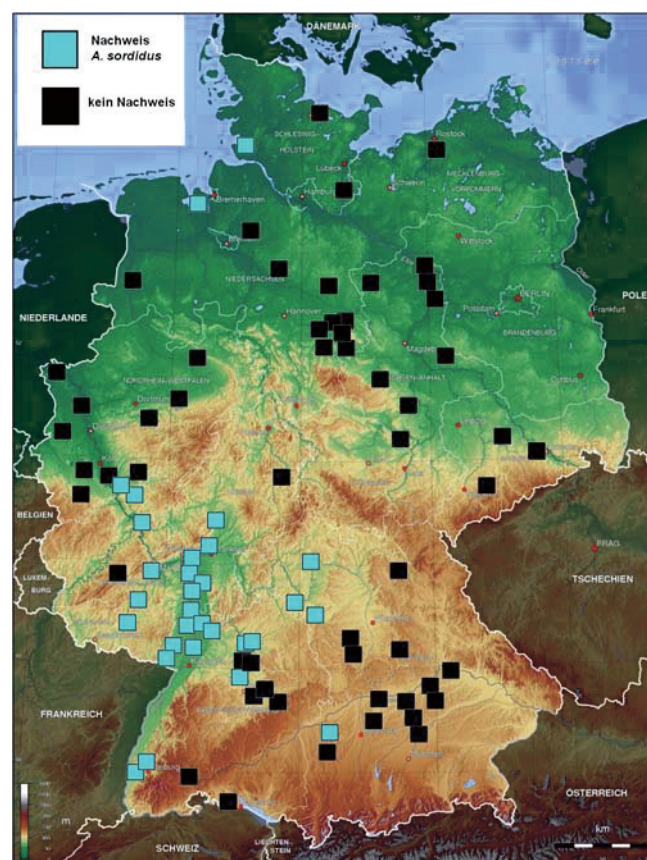


Fig. 2. Overview of the distribution of *Agriotes sordidus* in Germany according to the results of the wireworm and click beetle Monitoring 2011/2012 and data published (2007–2012).

Gabelbachergreut (Zusmarshausen) in Bavaria (two beetles in 2011, not sampled in 2012), Sehestedt in Lower Saxony (not sampled in 2011, 55 beetles in 2012) and Barlt in Schleswig-Holstein (not sampled in 2011, one beetle in 2012). The find in Barlt is the first find for Schleswig-Holstein, while the find in Gabelbachergreut is new for the region Schwaben within the federal state of Bavaria. The occurrence near Sehestedt represents the second find at this location and confirms a previous find from 2006 at that site (GOLLKOWSKI, 2007). At first glance this site (Fig. 4) seems an unlikely area for finding a southern species.

Specimens from all three isolated sites only matched *Agriotes sordidus* when determined morphologically with different keys (LOHSE, 1979; LAIBNER, 2000). But the individuals from the northern sites Sehestedt and Barlt on average turned out to be smaller and of a fainter brown color than the often relatively big, black specimens from the Upper Rhine valley, whereas the individuals from Gabelbachergreut visually matched those from the Upper Rhine (Fig. 3). That means visually the northern specimens bear a slight resemblance to *Agriotes rufipalpis* or *A. sputator* while the southern German specimens resemble a slightly elongated *A. obscurus*. Therefore beetles from the North German sites in Schleswig-Holstein and Lower Saxony were also determined using PCR next to the morphological determination, together with specimens from the location Pfungstadt-Eschollbrücken in the Upper Rhine valley for comparison (Fig. 5).

The newly isolated *Agriotes* specimens were found to be *Agriotes sordidus* by applying the PCR and the appropriate primers (STAUDACHER et al., 2011). These results confirmed the prior morphological species determination. No PCR fragments were found with the three larvae samples taken, when specific primers for *A. lineatus*, *A. sputator* and *A. obscurus* were applied (results not shown). To exclude any misinterpretation the *A. lineatus*, *A. sputator* and *A. obscurus* primers were applied with the

corresponding DNA isolated from the *Agriotes* isolates, respectively (Fig. 6). This confirmed that the imagines detected at the locations Barlt and Sehestedt are *Agriotes sordidus*.

Discussion

In the 1970ies *Agriotes sordidus* was reported in Germany only from the Kaiserstuhl area (Baden-Württemberg) in the Upper Rhine valley (LOHSE, 1979; ZEISING, 1984). This was based on the finding of a single male collected in Sasbach/Kaiserstuhl on 23 May 1972, published by ZEISING (1984) together with finds of other Elateridae considered remarkable for Middle Europe at that time. But over the last decades both a northward spread and a considerable increase in numbers have been observed, making *A. sordidus* today a common species in the Upper Rhine valley and spreading along some Rhine tributaries (BURGHAUSE and SCHMITT, 2011). This high frequency of *A. sordidus* observed in the Upper Rhine valley and the climatically mild valleys of Rhine tributaries was previously unsuspected, but was confirmed by the results of the click beetle monitoring. Considering that *A. sordidus* now has already passed through the Middle Rhine valley and reached Westerhausen at the geographic latitude of Bonn, a further spread into the Cologne lowland and along the lower Rhine seems possible, especially as this is also a climatically mild region and there are finds in further northern regions in the Netherlands and Germany. In the Cologne lowland a high proportion of sensitive vegetable crops are grown so that an increase in wireworm damage may occur here.

In the Netherlands the first record consists of two specimens of *Agriotes sordidus* (Illiger) that have been collected on a mudflat near Terneuzen, Zeeland, in April 2002 (VAN NUNEN, 2007). This is already further north than Westerhausen, the northernmost location along the



Fig. 3. Comparison of Northern and Southern German *Agriotes sordidus* males from left to right: Barlt (Schleswig-Holstein), Sehestedt (Lower Saxony); Wiesbaden-Nordenstadt (Hesse) Gabelbachergreut (Bavaria) (aedeagi not to scale). From these sites only Wiesbaden-Nordenstadt lies within the area of continuous distribution of *A. sordidus*. The aedeagus shows the curvature typical of *A. sordidus*, and all other morphological characters also point to *A. sordidus*, but there are marked visual differences between northern and southern specimens.



Fig. 4. Habitat of *A. sordidus* at a coastal site in Northern Germany (Sehestedt, Lower Saxony). The presence of the species at this site in 2006 and in 2012 indicates an established population.

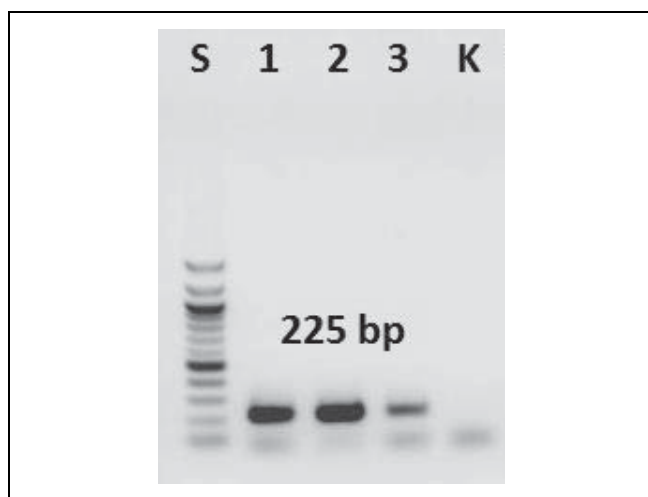


Fig. 5. PCR-assisted detection of imagines of *Agriotes sordidus* found in Northern Germany, in comparison with *A. sordidus* from a location from the Upper Rhine valley. To confirm the morphological identification via PCR as a second method, *A. sordidus* specific primers were used, generating a 225 bp fragment. The locations were 1 = Barlt (Schleswig-Holstein), 2 = Sehestedt (Lower Saxony), 3 = Pfungstadt-Eschollbrücken (Hesse, Upper Rhine valley region). K represents the water control and S the DNA size standard.

Rhine in Germany. This find is well documented with photos of the beetles and of male genitalia. According to the author it remains unknown if the occurrence is the result of a recent invasion or if the species has previously been overlooked. The specimens were found underneath typical saltmarsh plants (Sea Purslane *Halimione portulacoides*, Annual Seablite *Sueda maritima*), indicating a habitat under marine influence.

The most surprising recent records of this species, however, came from further north. One site was a coastal area in Lower Saxony in 2006 (GOLLKOWSKI, 2007), far away from the records in the Rhine valley. The continued existence of a population at this site was confirmed in 2012 with 55 males caught in a pheromone trap. This is another habitat under marine influence (foreland, saltmarsh). The northernmost location (Barlt, Schleswig-Holstein, a single trap-caught individual) is about 4–5 km from the sea and about 1 km from saltwater influenced greenland. These finds from 3 different sites hint to a possible coastal distribution in the Netherlands and northern Germany.

Verification of the morphological species determination appeared important as the northern beetles looked slightly different from the Upper Rhine beetles and slightly more akin to *Agriotes rufipalpis*. The remote possibility of an accidental introduction of a similarly looking species had to be excluded. For click beetles such a case had not yet been described. But in horticulture such introductions are known from *Otiorhynchus* weevils in plant nurseries, where the larvae are difficult to identify and PCR is used for larval identification (HIRSCH et al., 2010; SPRICK, 2009). To verify the morphological species determination of the click beetles from the coastal sites in Northern Germany, collected insects (wireworms and

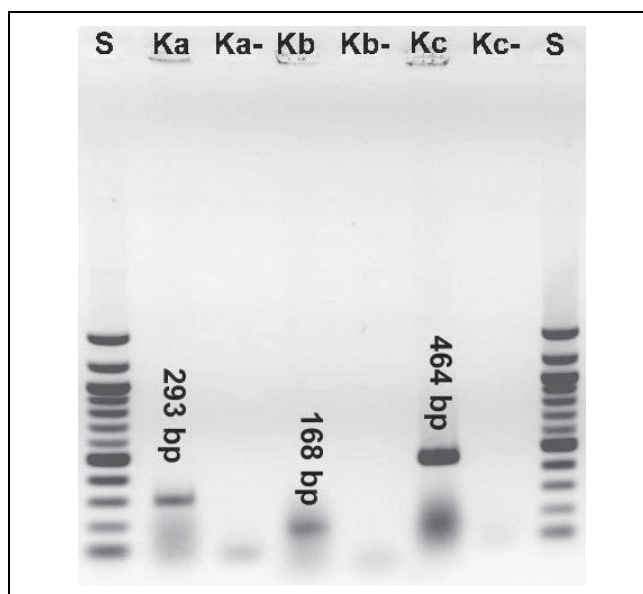


Fig. 6. To verify the specificity of the *A. sordidus* primer (STAUDACHER et al., 2011), morphologically determined larvae of *A. lineatus* (Ka), *A. sputator* (Kb) and *A. obscurus* (Kc) were used to determine the specificity of the detected *A. sordidus* beetle. The specificity of the primers for *A. lineatus* (Ka, 293 bp), *A. sputator* (Kb, 168 bp) and *A. obscurus* (Kc, 464 bp) were determined by using the corresponding DNA from morphologically determined *Agriotes* larvae. The obtained sizes of the fragments matched with the literature data (STAUDACHER et al., 2011). Ka-, Kb- and Kc- represent the water controls and S the DNA size standard.

click beetles) stored in 70%–80% ethanol, were also analyzed via PCR. After extracting the DNA from the ethanol conserved *Agriotes* imagines PCR amplifiable DNA was obtained, showing that DNA is still amplifiable in the PCR after the beetles had been caught with pheromone traps emptied in intervals of up to two weeks and had been kept in 70%–80% ethanol. Since the application of the *Agriotes sordidus* specific primers confirmed the morphological identification, a PCR detection with the applied primers can be considered an important tool for further confirmation of the identity of wireworms or click beetles.

The occurrence of *Agriotes sordidus* in or close to coastal, salt influenced habitats suggests two possibilities: Either the species is an accidental introduction in the region. Accidental wireworm transport with plants and the soil around their roots has been documented for North America. For example, the Canadian Nursery Certification Institute (CNCI) has 14 larval specimens of *Athous haemorrhoidalis* intercepted in shipments of ornamental trees and bushes to Canadian ports from 1961–1963 together with a few larvae of other species (DOUGLAS, 2011). This may also be a possible pathway for *A. sordidus* to northern Germany as the Ammerland region close to Oldenburg has a high concentration of plant nurseries at a distance of about 20–40 km to the finds at Sehestedt.

Or the species may have colonized the North German sites via a coastal route from the Netherlands, independent from the recent spread in the Upper Rhine valley and Rhine tributaries. As *A. sordidus* occurs in a similar

coastal, salt influenced habitat in the Netherlands (VAN NUNEN, 2007) and the closely related south-eastern European species *Agriotes rufipalpis* has been found in the Slovak Republic by MERTLIK (1998) in saline habitats, there seems to exist some tolerance to salt in this and other *Agriotes* species. But *A. sordidus* is not known to be halophilic and occurs also in non-saline habitats, meaning that other factors might influence this species' distribution. For example, the close proximity of the North Sea influences the air temperature regime experienced on a saltmarsh, reducing temperature extremes. Additionally, water covering the marsh can significantly alter soil temperatures, with warmer conditions in autumn, a milder winter and a cooler spring (BOORMAN, 2003). Larvae of *A. sordidus* might benefit from these conditions, possibly explaining the surprising occurrence of the species at these sites. But if *A. sordidus* is restricted to this habitat type in the north, the species in Lower Saxony and Schleswig-Holstein might not develop a similar economically important pest status as those populations in the Upper Rhine valley. To clarify this, adults will be monitored at additional coastal and inland sites, and a survey for larvae in the known saltmarsh habitat will be performed in 2013.

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