Occurrence of Agriotes *wireworms in Austrian agricultural land*

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Occurrence of *Agriotes* wireworms in Austrian agricultural land

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Abstract *Agriotes* wireworms (Coleoptera: Elateridae) are abundant soil-dwelling herbivores which can inflict considerable damage to field crops. In Europe up to 40 species occur, differing in their ecology and pest status. Their distribution in the larval stage, however, has rarely been assessed because of the considerable effort in collecting wireworms and the difficulties in identifying them to species-level. Here, we examined the occurrence of *Agriotes* wireworms in Austrian agricultural land with regard to their association with climatic and soil parameters. Using a molecular identification system, 1348 field-collected larvae from 85 sites were identified to species-level. Three species, *Agriotes obscurus*, *Agriotes brevis*, *Agriotes ustulatus*, and two that could not be discerned molecularly (*Agriotes lineatus* and *Agriotes proximus*), were assigned to two ecological groups: (i) *A. brevis/A. ustulatus*, found in areas with a warmer, drier climate and alkaline soils, and (ii) *A. obscurus/A. lineatus/proximus* which occur mainly at higher altitude characterised by lower temperatures, higher precipitation and acidic, humus-rich soils. *Agriotes sputator* was abundant throughout Austria, confirming its euryoecious nature. Only one larva of *Agriotes litigiosus* was found, prohibiting further analysis. These data contribute to a characterisation of species-specific traits in *Agriotes* larvae in agricultural land, an important prerequisite to develop efficient control strategies for these wireworms.

Keywords Elateridae · Pest control · Molecular identification · *Adrastus* spp. · Climatic factors · pH

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A contribution to the Special Issue on Ecology and Control of Wireworms

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Introduction

The larvae of *Agriotes* click beetles (Coleoptera: Elateridae), also referred to as wireworms, are abundant soil-dwelling insects representing the long-lived stage (i.e. 3–5 years, Dobrovolsky 1970). They are predominantly herbivorous (Traugott et al. 2008) and serious agricultural pests, attacking field crops such as potatoes and maize (Hill 1987; Parker and Howard 2001). Throughout Europe up to 40 *Agriotes* species occur (Cate 2007); some of them have been studied in detail (e.g. Furlan 1998, 2004; Kabanov 1975; Langenbuch 1932; Traugott et al. 2008), revealing differences in species-specific traits such as phenology, feeding behaviour and preferred environmental conditions. Still, for most *Agriotes* species, no precise data of their pest status and actual distribution in agricultural land are available which hampers the development of efficient pest control strategies (Parker and Howard 2001). In terms of their control, trap cropping offers a great potential (Vernon et al. 2000, 2005), yet it remains unknown whether species-specific dietary choices might influence the attraction of intercropped plant species. Similarly, the efficacy of entomopathogenic fungi and nematodes can vary amongst different *Agriotes* species (Jossi et al. 2008). The success of biofumigation also depends on species-specific traits (Furlan et al. 2010). In addition, behavioural as well as physiological differences between species are likely to affect the impact of soil-applied insecticides (Vernon et al. 2008).

Historically, few studies have focussed on *Agriotes* wireworm occurrence and species composition in agricultural land (Alekhin 1973; Jossi and Bigler 1997; Landl et al. 2010). Most studies have targeted adult male beetles as these can be easily collected in pheromone traps (e.g. Furlan and Tóth 2007; Subchev et al. 2005; Vernon and Tóth 2007). Although monitoring via pheromone trapping provides an effective means to collect adult male beetles (Tóth et al. 2003), there are constraints to this approach: for example, the long-lived larval population in the soil is not necessarily reflected by adult catches (Hicks and Blackshaw 2008; Landl et al. 2010). Moreover, pheromones are not available for all *Agriotes* species, rendering the adult species spectrum incomplete. Extensive field surveys of wireworms, on the other hand, entail time-consuming and laborious soil sampling and it is difficult to morphologically identify *Agriotes* wireworms to species-level. The latter can be resolved by applying a recently developed molecular identification system (Staudacher et al. 2011), which offers a reliable tool for identifying and monitoring *Agriotes* larvae typically found in Central Europe.

In Austria 14 *Agriotes* species have been identified (Cate 2007), yet it is unknown which species are most widespread and harmful in agricultural land and how the occurrence of these species is characterised by environmental factors. The present study had two aims: (i) to examine the species-specific occurrence of *Agriotes* wireworms in Austrian agricultural land and (ii) to analyse how the species requirements differ with regard to climatic and soil parameters.

Materials and methods

Collection of wireworms

Between 2007 and 2009, *Agriotes* larvae were collected in the course of numerous local studies at 85 agricultural sites throughout Austria (ESM 1). Several regions were chosen to cover the broad spectrum of environmental parameters present in Austria: the eastern foothills of the Alps and the periphery of the Pannonian Plain, the Austrian granite plateau located in the north (Bohemian Mass) and the alpine area in the western part of the country. Within regions the selection of the agricultural sites relied on monitored information about wireworm-induced damage. The sites included organic and conventional fields, planted with different crops (e.g. potato, maize) and grasslands (Table 1, ESM 2). The latter were investigated, as wireworms are typically linked to grassland in a rotation, where they can establish damaging populations, being present when a crop is sown (Blackshaw and Kerry 2008; Parker and Howard 2001). The wireworm sampling differed in mode and/or frequency based on the varying foci of the local studies. All wireworms were manually collected from a minimum of six soil samples or cereal baited traps per site and individually stored, either in 70–90% ethanol or frozen at −28°C.

Wireworm species identification

Aside from some *Agriotes ustulatus* larvae ($n = 54$, Table 1), which could be identified based on their distinct morphological characters (Klausnitzer 1994), all larvae investigated here were identified by molecular means as described in Staudacher et al. (2011). The remains of these larvae were stored in our laboratory at Innsbruck University and are available for further examination. The two species *Agriotes lineatus* and *Agriotes proximus* remained molecularly indistinguishable because of the low interspecific sequence divergence as reported earlier (Staudacher et al. 2011). DNA extracts which did not amplify in the diagnostic multiplex PCRs were sequenced to barcode these specimens.

The DNA barcoding revealed that several specimens which were assigned to the genus *Agriotes* during field sampling had a perfect match with sequences of *Adrastus* spp., an elaterid genus which is morphologically very similar to *Agriotes* spp. in the larval stage (Klausnitzer 1994). Hence, specific primers targeting *Adrastus* spp. were designed to reduce the number of specimens which needed to be sequenced. The newly developed primers Adr-spp-S540 (5′-AACTGACTTGTTCCTCTAATACTG-3′) and Adr-spp-A549 (5′-CGATCAAATGAAATTCCAAC-3′) amplify a 302 bp fragment of the mitochondrial cytochrome *c* oxidase subunit I gene. The specificity of this primer pair was tested against all Central European *Agriotes* species as well as against other elaterid species [e.g. *Agrypnus murinus* (L.), *Hemicrepidius niger* (L.)] and non-elaterid soil invertebrates (see list of non-target species in Staudacher et al. 2011). Each 10 µl PCR contained 1.5 µl of DNA extract, 0.375 U BioTherm™ *Taq* DNA Polymerase (Ares Bioscience, Köln, Germany), 1 µl 10× Reaction Buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs (Ares Bioscience), 1 µM of each primer, 5 µg bovine serum albumin (BSA) and 3.325 µl PCR-grade RNase-free water (Qiagen, Hilden, Germany). The thermocycling program (executed on a Mastercycler Gradient, Eppendorf, Hamburg, Germany) consisted

Table 1 *Agriotes* larvae collected in Austrian agricultural land ('*E*', '*N*' and '*W*' indicate eastern, northern, and western [i.e. alpine] part, of the country) and identified by molecular means as described in Staudacher et al. (2011) (numbers in parenthesis represent morphologically identified *Agriotes ustulatus* larvae)

Region	No. of sites	∑ *Agriotes* larvae	*Agriotes obscurus* (L.)	*Agriotes sputator* (L.)	*Agriotes brevis* Candèze	*Agriotes ustulatus* (Schaller)	*A. lineatus* (L.)/ *A. proximus* Schwarz	*Agriotes litigiosus* (Rossi)
Pannonian Plain (E)	5	54		3	35	16 (14)		
Marchfeld (E)	9	150	1	5	131	13 (1)		
Wein-/Mostviertel (E-N)	27	203	37	60	51	55 (39)		
Wald-/Mühlviertel (N)	14	126	34	78	11		3	
Inn-/Hausruckviertel (N)	9	93	52	22	11	2	5	1
Tyrol (W, Alps)	21	616	465	151				
Σ	85	1242	589	319	239	86 (54)	8	1

of an initial activation step of 2 min at 94°C, followed by 35 cycles of 20 s at 94°C, 30 s at 50°C, 45 s at 72°C and a 2 min final extension at 72°C. PCR products were electrophoresed on 1.5% agarose gels stained with GelRed™ (Biotium, Hayward, USA) and visualized under UV light.

Environmental factors and statistical analyses

The distribution of *Agriotes* species within the Austrian sampling sites was displayed on maps created in ESRI® ArcGIS 9.3 (ESRI, Redlands, USA). Geographic (ESRI Data & Maps 2006, Media Kit) and climatic parameters (WorldClim, Hijmans et al. 2005) were obtained from freely accessible data layers. Presence/absence data of the different species and not their abundances were used for statistical analyses as the sampling was done in a qualitative manner. One-way ANOVAs were used to evaluate environmental factors, i.e. altitude, annual mean temperature and annual precipitation, for significant differences between the sites. All sampling sites located up to 8.2 km distance between each other were pooled, as they share similar climatic conditions, reducing the 85 sites to 25 areas. These analyses were conducted in PASW Statistics 18 (SPSS Inc., Chicago, USA).

Multivariate analyses were performed with CANOCO for Windows 4.5 (ter Braak and Šmilauer 2002) considering every single site. Canonical correspondence analysis (CCA) was used to examine how species distribution was related to climatic parameters (i.e. altitude, annual mean temperature, annual precipitation, temperature/precipitation seasonality, winter/summer temperature extremes, winter/summer precipitation and isothermality). Likewise, site-specific soil parameters (pH, lime concentration, water-holding capacity and water permeability, humus content, agricultural suitability and land value) obtained from soil maps from the eBOD database ('Digitale Bodenkarte von Österreich, BFW') were correlated with the occurrence of *Agriotes* species. Statistical significance of axes was tested using Monte Carlo permutation tests (999 permutations under the reduced model). Because the sampling design was not laid out for a comparison between organic and conventional sites or between different crops, these variables were not contrasted in our analysis.

Results

Agriotes larvae from Austrian agricultural land

Within the 85 sampling sites, six *Agriotes* species comprising 1,242 individuals were identified: *A. obscurus*, *A. sputator*, *A. brevis*, *A. ustulatus*, *A. litigiosus* and the two indistinguishable species *A. lineatus* and *A. proximus* (Table 1, ESM 1). Within 50% of the investigated sites, more than one *Agriotes* species was recorded; at three sites a maximum of four species co-occurred. In addition, 92 larvae of *Adrastus* spp. were identified via the *Adrastus*-specific PCR and DNA barcoding. Only 14 specimens of the field-collected wireworms did not provide amplifiable DNA.

Agriotes obscurus was most frequently found ($n = 589$), especially in the alpine area (Tyrol), followed by *A. sputator* ($n = 319$). *Agriotes brevis* ($n = 239$) and *A. ustulatus* ($n = 86$), however, were only found in the northern and eastern parts of the country. *Agriotes lineatus/proximus* was occasionally found in lower numbers and *A. litigiosus* was represented by a single larva collected in the Hausruckviertel (Fig. 1; Table 1).

Climatic parameters and the occurrence of *Agriotes* larvae

Significant differences in the altitudinal occurrence of the *Agriotes* wireworms were found for five species (*F*-ratio

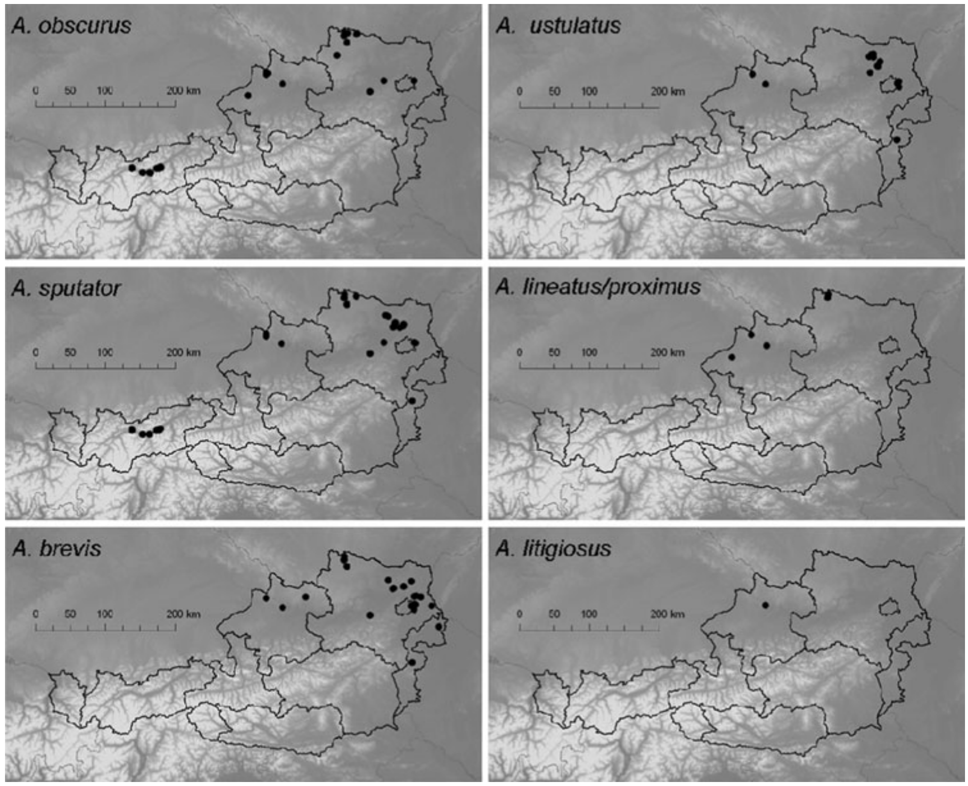

Fig. 1 Agricultural sites in Austria where different *Agriotes* wireworms were found

$= 2.924$, $P = 0.03$; single count for *A. litigiosus* not considered): *A. obscurus* larvae were collected on sites with a mean altitude of 481.8 m a.s.l., which was higher compared to the mean altitude where wireworms of *A. brevis* and *A. ustulatus* were found (304.7 and 279 m, respectively). The distributional centre of *A. lineatus/proximus* and *A. sputator* larvae was around 430 m a.s.l. (Fig. 2a). In terms of climate-related species occurrence, only annual precipitation values were found to be significantly different between species (*F*-ratio $= 2.593$, $P = 0.047$). Annual precipitation at the preferred sites of *A. lineatus/proximus* and *A. obscurus* larvae averaged 953.8 and 833.5 mm, respectively. The larvae of *A. brevis*, *A. ustulatus* and *A. sputator* occurred in regions with lower annual precipitation, averaging between 715.1, 731.4 and 765.3 mm, respectively (Fig. 2b). The preferred sites of *A. lineatus/proximus* and *A. obscurus* were also characterised by lower annual mean temperatures (averaged 8.15 and 8.36°C, respectively), whereas larvae of *A. brevis*, *A. ustulatus* and *A. sputator* preferred sites with higher annual mean temperatures (averaged 8.94, 9.13 and 8.7°C, respectively) (Fig. 2c).

Similar relationships were found when performing a CCA (Fig. 3): the arrangement of the five *Agriotes* species along the first axis (eigenvalue $= 0.408$, *F*-ratio $= 18.733$, $P = 0.001$) clearly corresponds to the altitudinal gradient. The occurrence of *A. obscurus* and *A. lineatus/proximus* correlates positively with rising altitude, accompanied by increasing annual precipitation (mean, summer and winter values, respectively). In contrast, *A. brevis* and *A. ustulatus* tend to be more frequent with increasing annual mean temperature and temperature seasonality as well as maximum temperature in the warmest month and minimum temperature in the coldest month (extremes). The second axis (eigenvalue $= 0.194$) separates *A. lineatus/proximus* from all the other species because of its occurrence on sites characterised by high precipitation during the winter months.

Soil parameters and the occurrence of *Agriotes* larvae

The CCA of the *Agriotes* species distribution and different soil parameters (Fig. 4) resulted in a separation along the first axis (eigenvalue $= 0.190$, *F*-ratio $= 7.972$, $P = 0.003$) according to soil pH. This gradient corresponds to a separation of *A. ustulatus* (sites with higher pH values/lime concentrations) from *A. brevis/A. sputator* and *A. obscurus/A. lineatus/proximus* (sites with lower pH). The second axis (eigenvalue $= 0.090$) roughly separates the agricultural sites according to water balance-related parameters. Here, the occurrence of *A. brevis* larvae correlates with increased water permeability of the soil compared to the other species. *Agriotes obscurus* and *A. lineatus/proximus* wireworms were found more frequently in humus-rich soils, the latter correlating positively with higher water-holding capacity.

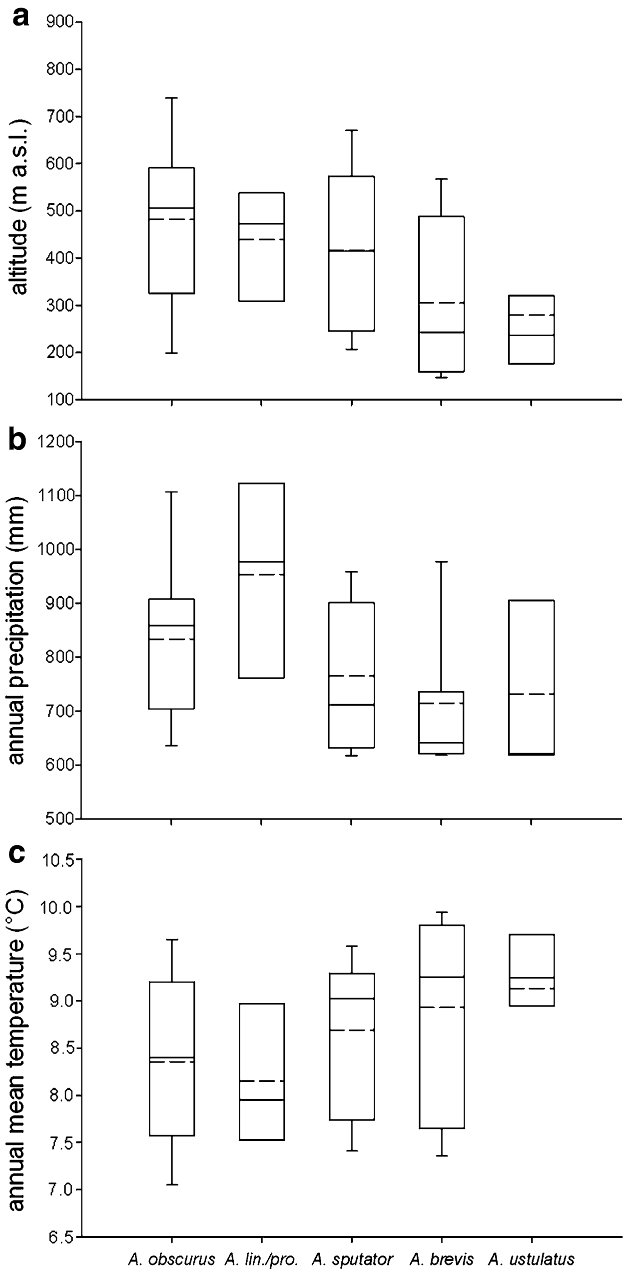

Fig. 2 Occurrence of five *Agriotes* species (*Agriotes lineatus/proximus* abbreviated as *A. lin./pro.*) in Austrian agricultural land with regard to **a** altitude, **b** annual precipitation and **c** annual mean temperature. *Box-plots* display the median, 25 and 75th percentiles (*line*, *box*), arithmetic mean (*dashed line*) and the 10 and 90th percentiles (*whiskers*). Number of areas where species occur are: 14 (*A. obscurus*), 4 (*A. lin./pro.*), 16 (*A. sputator*), 15 (*A. brevis*) and 7 (*A. sputator*)

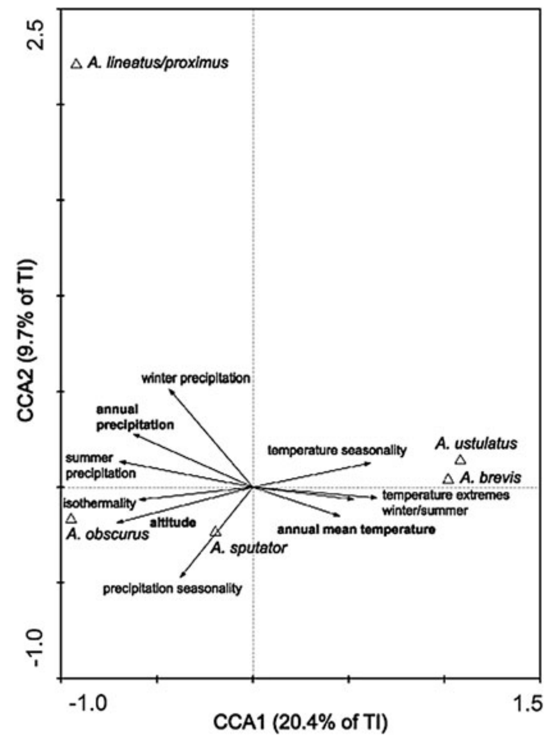

Fig. 3 Canonical correspondence analysis of climatic parameters (vectors) and five *Agriotes* species (indicated by *triangles*) based on their occurrence in agricultural land in Austria ($n = 85$). Ordination biplot for 1st (CCA1) and 2nd axis (CCA2); variation explained by *axes* is given as a percentage of total variation (total inertia, TI)

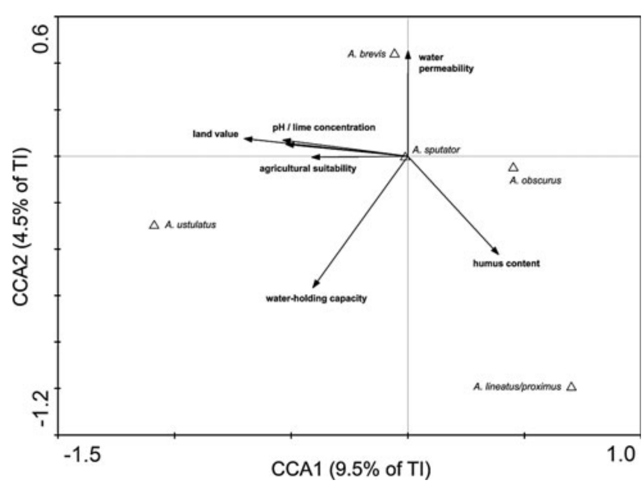

Fig. 4 Canonical correspondence analysis of soil parameters (vectors) and five *Agriotes* species (indicated by *triangles*) based on their occurrence in agricultural land in Austria ($n = 85$). Ordination biplot for 1st (CCA1) and 2nd axis (CCA2); variation explained by *axes* is given as a percentage of total variation (TI)

Discussion

This study represents the first extensive field survey of *Agriotes* wireworms in Austrian agricultural land. We detected six out of 14 *Agriotes* species known to occur in Austria. *Agriotes obscurus*, *A. sputator*, *A. brevis* and *A. ustulatus* were found to be the most abundant species, confirming their importance in arable land and corroborating observations from other parts of Europe (e.g. Furlan and Tóth 2007; Jossi and Bigler 1997; Tóth 1984). The remaining species absent in this study are either rare or do not occur in arable fields and exposed grasslands.

By collecting a large number of wireworms throughout the country we were able to assign four *Agriotes* species (*A. lineatus*/*A. proximus* as a group) to two ecological groups, each showing a preference for distinct environmental conditions. One group (*A. brevis* and *A. ustulatus*) dominated in areas with a warmer and drier climate than the other group (*A. lineatus/proximus* and *A. obscurus*) which occurred mainly at higher altitudes, characterised by lower temperatures and higher precipitation. Aside from these species, *A. sputator* larvae were found to be widespread, confirming the species' euryoecious nature (Franz 1974; Wörndle 1950). Likewise, Alekhin (1973) showed that *A. sputator* occurs in six different climatic regions in Russia, ranging from the forest-steppe zone to the Volga delta. The single count for *A. litigiosus* prohibited any further analysis in the present study.

The majority of the sites where larvae of *A. obscurus* occurred were located in the western, alpine region (Tyrol) and higher reaches of Upper and Lower Austria. This fits the previously described distribution of this species (adult beetles) in the northern parts of Austria and higher altitudes (Franz 1974; Jossi and Bigler 1997; Wörndle 1950), also indicating that this species generally favours lower temperatures. Previous studies showed that *A. obscurus* was, apart from grasslands, very abundant in maize/potato fields in Tyrol (Schallhart et al. 2009; Traugott et al. 2008). Langenbuch (1932) showed that late instar *A. obscurus* wireworms can survive several hours at −14°C and are frost resistant over several weeks. Literature data indicate that the preferred environmental conditions of *A. lineatus* are comparable to *A. obscurus* (Kabanov 1975; Langenbuch 1932; Schimmel 1989; Tóth 1984). Indeed, in the present study, larvae of *A. lineatus/proximus* were found only in northern Austria (Waldviertel–Innviertel), characterised by high mean annual precipitation (see also Landl et al. 2010). *Agriotes lineatus* is known to be hydrophilic and especially moisture-resistant (Langenbuch 1932), and according to Kabanov (1975) these wireworms are able to survive flooding over several days. The current data indicate that *A. lineatus/proximus* and *A. obscurus* larvae have similar preferences regarding the examined soil parameters, i.e. soil acidity and humus-richness.

Larvae of *A. brevis* and *A. ustulatus* were mostly restricted to the eastern parts of Austria, ranging from Upper Austria (Waldviertel–Weinviertel) to the periphery of the Pannonian plain. This result confirms the preference of *A. ustulatus* for warmer and drier climates described earlier (Furlan 1998). Previous investigations showed that this species mainly occurs in Southern/Central-Eastern Europe (Furlan and Tóth 2007; Franz 1974; Schimmel 1989; Wörndle 1950). Rusek (1972) and Franz (1974) suggested that *A. brevis* also favours higher mean annual temperatures and less precipitation. In the present study, this species was found more frequently in sandy, humus-poor soils.

So far, only a few investigations on *Agriotes* wireworm occurrence have been conducted in Austria (Brunner et al. 2005; Landl et al. 2010) which depended on laborious and uncertain morphological larval identification. The present study demonstrates the applicability of the molecular identification protocol for large scale surveys: 92% of all collected wireworms were directly identified via multiplex PCRs, which proved to be highly suitable to cover the key *Agriotes* species present in agricultural land in Central Europe. Furthermore, we provide new *Adrastus* spp. primers, allowing the differentiation of these wireworms (e.g. *Adrastus montanus* (Scopoli)) from the very similar looking *Agriotes* larvae. The new primers will be useful for studies dealing with other wireworm genera where identification to species level was not yet possible (Benefer et al. 2010; Parker and Seeney 1997; Samson and Calder 2003).

Conclusion

The present data provide both new insights into the occurrence of *Agriotes* larvae in Austria and a better characterisation of species-specific traits, i.e. information on environmental factors which drive the distribution of *Agriotes* larvae in agricultural land. As behavioural and phenological differences between species affect the efficacy of control strategies such as trap cropping or the use of entomopathogens and insecticides, these data will be helpful to develop species-specific control strategies.

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