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Original article (Orijinal araştırma)

Molecular identification and biodiversity of wireworm species, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae) in major potato cultivated areas of Türkiye[1]

Tel kurdu türlerinin, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae) Türkiye'nin başlıca patates ekim alanlarındaki biyoçeşitliliği ve moleküler tanımlanması

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Abstract

Wireworms, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae) are among the most harmful soil-borne insect pests and significantly reduce potato yields under heavy infestations. The presence of wireworm species on potatoes in potato growing areas is not fully known in the provinces of Türkiye. Therefore, this research aimed to identify wireworms molecularly and evaluate their biodiversity in potato growing ecosystems. Here, the first extensive field survey was carried out in Türkiye's principal potato-growing regions in 2019 and 2020 (Afyon, Bolu, İzmir, Kayseri, Konya, Niğde, and Sivas). Species identification of wireworms was performed using DNA barcoding approach based on the fragment of mitochondrial cytochrome c oxidase subunit I (COI). Samples were collected from 400 potato fields, and 510 larval specimens were obtained. The presence of wireworms was confirmed for Afyon, Bolu, Kayseri, Konya, and Sivas provinces, with an average prevalence of 13.5%, while no positive samples were recovered from Niğde and İzmir. *Agriotes sputator* (L.,1758) (Coleoptera: Elateridae) was the most prevalent species in surveyed areas. The Shannon index of wireworm species was found to be as 0.59, which implies a low degree of biodiversity of wireworms in potato fields.

Keywords: *Agriotes* spp., COI, Elateridae, potato, soil-borne pests

Öz

Tel kurtları, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae), en önemli toprak kaynaklı zararlı böcekler arasında yer almakta ve yoğun istilalarda patates üretiminde ciddi verim kayıplarına neden olmaktadır. Türkiye'de patates yetiştirilen alanlarda zararlı tel kurdu türlerinin varlığı tam olarak bilinmemektedir. Bu nedenle bu çalışma, patates yetiştiriciliği yapılan ekosistemlerde tel kurdu türlerinin moleküler olarak tanılanmasını ve biyoçeşitliliğini belirlemeyi amaçlamıştır. Bu çalışmada, 2019 ve 2020 yıllarında Türkiye'nin başlıca patates yetiştirilen bölgelerinde (Afyon, Bolu, İzmir, Kayseri, Konya, Niğde ve Sivas) kapsamlı ilk saha araştırması yapılmıştır. Tel kurtlarının tür tanılaması, mitokondriyal sitokrom c oksidaz Alt Ünite I (COI) geni dizisine dayalı DNA barkodlaması kullanılarak yapılmıştır. Örnekler, 400 adet patates tarlasından toplanmış ve 510 adet larva örneği elde edilmiştir. Afyon, Bolu, Kayseri, Konya ve Sivas illerinde tel kurtlarının yaygınlığı ortalama %13,5 olarak belirlenirken, Niğde ve İzmir illerinden pozitif örnek elde edilmemiştir. *Agriotes sputator* (L., 1758) (Coleoptera: Elateridae) türünün örnekleme yapılan alanlarda en yaygın tür olduğu belirlenmiştir. Tel kurdu türlerinin Shannon indeksi 0.59 olarak bulunmuş olup, patates tarlalarında tel kurtlarının biyolojik çeşitliliğinin düşük olduğu belirlenmiştir.

Anahtar sözcükler: *Agriotes* spp., COI, Elateridae, patates, toprak kaynaklı zararlılar

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Introduction

The larvae of click beetles, *Agriotes* spp. Eschscholtz, 1829 (Coleoptera: Elateridae), known as wireworms, are mostly herbivorous and regarded as one of the most serious pests of crops in the world (Traugott et al., 2015; Poggi et al., 2021). Although the adults of the *Agriotes* spp. feed on the leaves of plants, they rarely cause economic losses. However, the larvae of wireworms are common soil-dwelling insects that seriously harm the roots and seeds of many different crops during their larval development (Ritter & Ritcter, 2013). The damaged plants are generally exposed to secondary infections by various soil-borne pathogens and lose their marketable quality (Susurluk, 2008; Keiser et al., 2012; Traugott et al., 2015). Wireworm damage has increasingly been a problem for Turkish potato growers (Gülperçin & Tezcan, 2021; Kabalak & Sert, 2021). As an underground crop, potato tubers are particularly susceptible to the larval damage of *Agriotes* spp., and yields and quality can drop severely even at low population densities (Parker & Howard, 2001; Furlan, 2014).

Among over 9,000 wireworm species identified worldwide, over 39 species of wireworms are known as potato pests (Kroschel et al., 2020), and nine species; *Agriotes brevis* Candèze, 1863, *Agriotes lineatus* L., 1767, *Agriotes litigiosus* Rossi, 1792) *Agriotes obscurus* L., 1758, *Agriotes proximus* Schwarz, 1891, *Agriotes rufipalpis* Brullé, 1832, *Agriotes. sordidus* Illiger, 1807, *Agriotes. sputator* L., 1758 and *Agriotes. ustulatus* Schaller, 1783 are considered the most devastating ones in Europe (Furlan & Tóth, 2007; Furlan et al., 2021). To date, 483 wireworm species have been reported from various agricultural lands in Türkiye (Kabalak, 2018; Gülperçin & Tezcan, 2021; Kabalak & Sert, 2021). However, majority of these studies are based on morphological characteristics to identify wireworm species. The morphological identification is quite challenging due to the lack of clear distinguishing characters between species (Benefer et al., 2013; Andrews et al., 2020). The mitochondrial cytochrome c oxidase subunit I (COI) provides more accurate identification of the wireworm species and has also been used to construct the phylogeny of the wireworm specimens (Staudacher et al., 2013; Etzler et al., 2014; Zhang et al., 2019; Andrews et al., 2020). Accurately identifying pest species is the first step to achieving a more effective management strategy in integrated pest management (IPM) practices since damage risk and economic threshold levels may vary with the different wireworm species (Furlan, 2014; Furlan et al., 2021). The Shannon diversity index estimates species diversity within a community, and, may indicate the diversity and relative abundance of species in a given community (Shannon, 1948). The main goal of this study was to identify the biodiversity, prevalence, and species composition of wireworms in the major potato-cropping agroecosystems of Türkiye.

Material and Methods

Sampling of *Agriotes* spp.

In 2019 and 2020, soil samples were taken from 400 different potato fields in Afyon, Bolu, İzmir, Kayseri, Konya, Niğde, and Sivas provinces in Türkiye. Sampling was performed when the soil temperature reached 10°C between May and June. The potato plants showing wilt, chlorosis, and stunted growth were located in each sampling area. Using a shovel, the soil was dug up from at least ten separate sites in sampling fields to obtain the larval stages of wireworm species. The samples were taken from 20 cm in diameter and 10 cm in depth from each point (Furlan & Tóth, 2007). Soil samples were sieved through 300 µm to uncover the larval stages of wireworms. The collected wireworm larvae were placed individually into plastic containers containing 95% ethanol and labelled with a specimen code. The samples were then kept in a cooler bag (approximately 15°C) until they were transported to the laboratory.

Molecular analyses

Total DNA extractions were performed using the tissue in the legs and abdominal sections of the larval specimens. Amplification of COI gene fragment were conducted using the polymerase chain reaction (PCR) amplification Kit (Cat. No. PCR-111S; Jena Bioscience GmbH, Jena, Germany) according to the manufacturer's instructions with a universal primer pair of LCO1490 5′-GTCAACAAATCATAAAGATATTGG-3′ and HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer et al., 1994). PCR cycle conditions were 94°C for 5 min; 35 cycles of 94°C for 30 s, 54°C for 45 s and 72°C 60 s; and finally, 72°C for 60 s in a T100 thermal cycler (Bio-Rad, Hercules, CA, USA). Sequencing of the PCR amplicons were carried out using one larval specimen for each population. PCR amplicons were purified and bidirectionally sequenced with the same primers by the Macrogen company (Seoul, South Korea).

The resultant sequences were processed using the MegAlign module of DNASTAR v7.1.0 (DNASTAR Inc., Madison, Wisconsin, USA). The obtained sequences were compared to the GenBank database (http://www.ncbi.nlm.nih.gov/genbank) by using the Basic Local Alignment Search Tool (BLAST), and representative species sequences for provinces were deposited in GenBank (*Agriotes sputator*: OP630854, OP630855, OP630856, and OP630857; *A. rufipalpis*: OP630858). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei, 1987). The optimal tree with the sum of branch length = 0.54149612 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. *Meloidogyne incognita* (Kofoid & White, 1919) was included as outgroups, and sequences were extracted from GenBank. The analysis involved 71 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 503 positions in the final dataset. Evolutionary analyses were conducted in MEGA 7 (Kumar et al., 2016).

Diversity Index

The Shannon Diversity Index (H) is a method for measuring diversity of species in a community and calculated as: $H = -\Sigma pi * \ln (pi)$. "Σ": A Greek symbol meaning "sum"; "ln": Natural log; "pi": (Shannon, 1948). The higher the "H" value, the higher the species diversity is in a particular community. The lower the "H" value, the lower the diversity is in a given community (Shannon, 1948). Shannon diversity index (H) is classified according to the following grouping: low ($H < 2$); medium ($2 < H < 4$); and high ($H > 4$) species (Lumeran, 2019). In this study, this index was used to describe variations in wireworm populations and discriminate the biodiversity levels of wireworm species.

Results and Discussion

In total, 510 larvae were collected from 400 samples taken from different potato cultivation locations of sampled provinces. The highest occurrence rate was obtained by Sivas, followed by Kayseri and Bolu provinces (Table 1).

Table 1. Wireworm-positive soil samples collected from potato cultivation areas of seven provinces of Türkiye

Province	Wireworm-positive samples	Wireworm-negative samples	Total	Occurrence rate (%)
Afyon	6	64	70	8.5
Konya	12	58	70	17.1
İzmir	0	60	60	0.0
Sivas	14	36	50	28.0
Bolu	12	38	50	24.0
Kayseri	10	40	50	25.0
Niğde	0	50	50	0.0
Total	**54**	**346**	**400**	**13.5**

No wireworm specimens have been found in the potato growing areas of Niğde and İzmir provinces (Table 2). The majority of the specimens were identified as *A. sputator* (90%), and the rest of the specimens were *A. rufipalpis* (10%), which were encountered only in Afyon (Figure 1). *A. sputator* was the most common species and obtained from all provinces except Afyon (Tables 1 & 2).

Table 2. The list of locations where wireworms were detected

No	Specimen name	Species	No. of larvae extracted	GPS Coordinates
1	AF-1	*Agriotes rufipalpis*	7	38°41’18”K 31°02’47”D
2	AF-2	*Agriotes rufipalpis*	11	38°43’43”K 31°02’30”D
3	AF-3	*Agriotes rufipalpis*	7	38°42’12”K 30°38’37”D
4	AF-4	*Agriotes rufipalpis*	10	38°44’39”K 30°41’14”D
5	AF-6	*Agriotes rufipalpis*	9	38°35’23”K 31°02’52”D
6	AF-8	*Agriotes rufipalpis*	7	38°35’36”K 30°58’19”D
7	BOL-1	*Agriotes sputator*	16	40°45’10’’K 31°32’98’’D
8	BOL-2	*Agriotes sputator*	7	40°45’12’’K 31°32’90’’D
9	BOL-3	*Agriotes sputator*	53	40°45’07’’K 31°32’00’’D
10	BOL-4	*Agriotes sputator*	9	40°45’04’’K 31°33’00’’D
11	BOL-5	*Agriotes sputator*	24	40°45’14’’K 31°33’11’’D
12	BOL-6	*Agriotes sputator*	11	40°45’13’’K 31°33’13’’D
13	BOL-8	*Agriotes sputator*	7	40°45’56’’K 31°37’11’’D
14	BOL-10	*Agriotes sputator*	4	40°45’83’’K 31°37’19’’D
15	BOL-12	*Agriotes sputator*	13	40°46’01’’K 31°37’19’’D
16	BOL-14	*Agriotes sputator*	4	40°46’41’’K 31°37’32’’D
17	BOL-16	*Agriotes sputator*	7	40°46’39’’K 31°37’41’’D
18	BOL-18	*Agriotes sputator*	16	40°46’88’’K 31°37’74’’D
19	KON-1	*Agriotes sputator*	5	37°35’21”K 32°48’40”D
20	KON-2	*Agriotes sputator*	6	37°36’17”K 32°49’50”D
21	KON-3	*Agriotes sputator*	4	37°36’43”K 32°50’00”D
22	KON-4	*Agriotes sputator*	3	37°32’30”K 32°49’40”D
23	KON-5	*Agriotes sputator*	12	37°36’24”K 32°44’03”D
24	KON-6	*Agriotes sputator*	8	37°37’16”K 32°42’52”D
25	KON-10	*Agriotes sputator*	6	38°00’52”K 32°00’38”D
26	KON-12	*Agriotes sputator*	17	38°01’18”K 32°00’49”D
27	KON-14	*Agriotes sputator*	14	38°00’54”K 31°59’25”D
28	KON-16	*Agriotes sputator*	8	38°00’49”K 31°59’59”D
29	KON-18	*Agriotes sputator*	12	37°29’54”K 34°01’34”D
30	KON-20	*Agriotes sputator*	15	37°29’06”K 34°00’07”D
31	SİV-1	*Agriotes sputator*	9	39°10’29”K 36°05’04”D
32	SİV-2	*Agriotes sputator*	13	39°11’26”K 36°05’06”D
33	SİV-3	*Agriotes sputator*	10	39°12’27”K 36°05’59”D
34	SİV-4	*Agriotes sputator*	4	39°13’29”K 36°07’05”D
35	SİV-5	*Agriotes sputator*	6	39°16’19”K 36°11’30”D
36	SİV-6	*Agriotes sputator*	9	39°16’43”K 36°12’16”D
37	SİV-7	*Agriotes sputator*	6	39°17’24”K 36°14’18”D
38	SİV-14	*Agriotes sputator*	7	39°18’00”K 36°23’16”D
39	SİV-16	*Agriotes sputator*	11	39°18’07”K 36°18’53”D
40	SİV-18	*Agriotes sputator*	9	39°19’07”K 35°56’40”D

Table 2. Continued

No	Specimen name	Species	No. of larvae extracted	GPS Coordinates
41	SİV-20	*Agriotes sputator*	7	39°07'13"K 36°05'18"D
42	SİV-22	*Agriotes sputator*	9	39°07'20"K 36°04'51"D
43	SİV-24	*Agriotes sputator*	6	39°12'13"K 36°05'36"D
44	SİV-26	*Agriotes sputator*	8	40°09'30"K 38°07'45"D
45	KAY-1	*Agriotes sputator*	12	38°23'09"K 35°29'37"D
46	KAY-2	*Agriotes sputator*	6	38°21'35"K 35°28'19"D
47	KAY-3	*Agriotes sputator*	5	38°21'19"K 35°28'08"D
48	KAY-4	*Agriotes sputator*	9	38°20'28"K 35°27'49"D
49	KAY-5	*Agriotes sputator*	7	38°19'18"K 35°26'36"D
50	KAY-6	*Agriotes sputator*	3	38°18'58"K 35°26'17"D
51	KAY-8	*Agriotes sputator*	4	38°18'11"K 35°25'58"D
52	KAY-10	*Agriotes sputator*	5	38°16'49"K 35°25'15"D
53	KAY-12	*Agriotes sputator*	6	38°14'32"K 35°26'01"D
54	KAY-14	*Agriotes sputator*	7	38°21'11"K 35°23'26"D

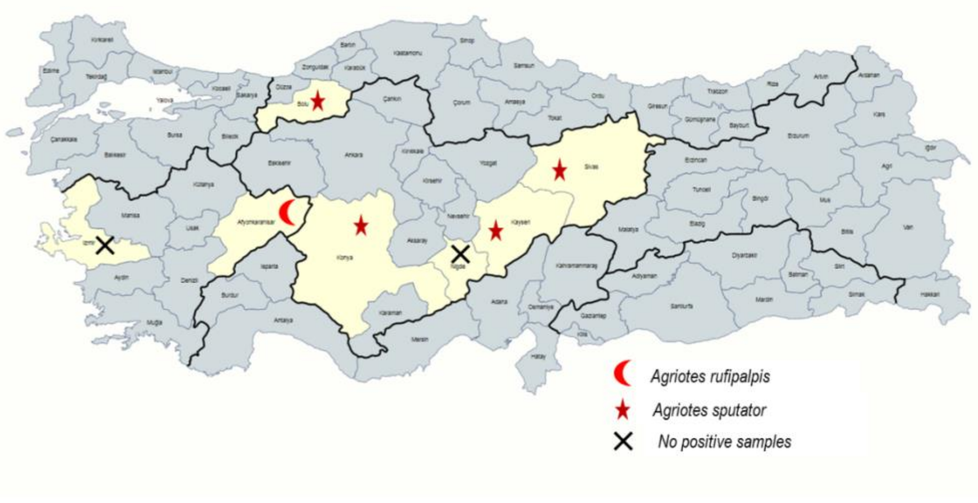

Figure 1. The map of the surveyed locations of wireworm species in potato-growing areas of Türkiye.

The Shannon diversity index (H) was determined to be H= 0.5915 for the wireworm species in potato growing areas. The occurrence of the low "H" index (H < 2) indicates a lack of variation (Table 3).

Table 3. Shannon diversity index "H"of wireworm species in potato fields

Wireworm species	Number	Pi*	ln (pi)	pi*ln (pi)
Agriotes rufipalpis	368	0.721569	-0.32633	-0.23547
Agriotes sputator	142	0.278431	-1.27858	-0.356
Total	510	1	-1.60491	-0.59147
			H	0.59147

* H = -Σpi * ln (pi). "Σ": A Greek symbol meaning "sum"; "ln": Natural log; "pi": proportion of the entire population. Multiplied with negative one.

DNA was extracted from one specimen of each population, and all the extracted specimens were successfully sequenced. Phylogenetic analysis comprised 60 nucleotide sequences and was performed using the Maximum Likelihood approach based on the General Time Reversible model. Beside the branches, the bootstrap test's percentage of duplicate trees where the related taxa are grouped (1000 repetitions) is displayed (Figure 2).

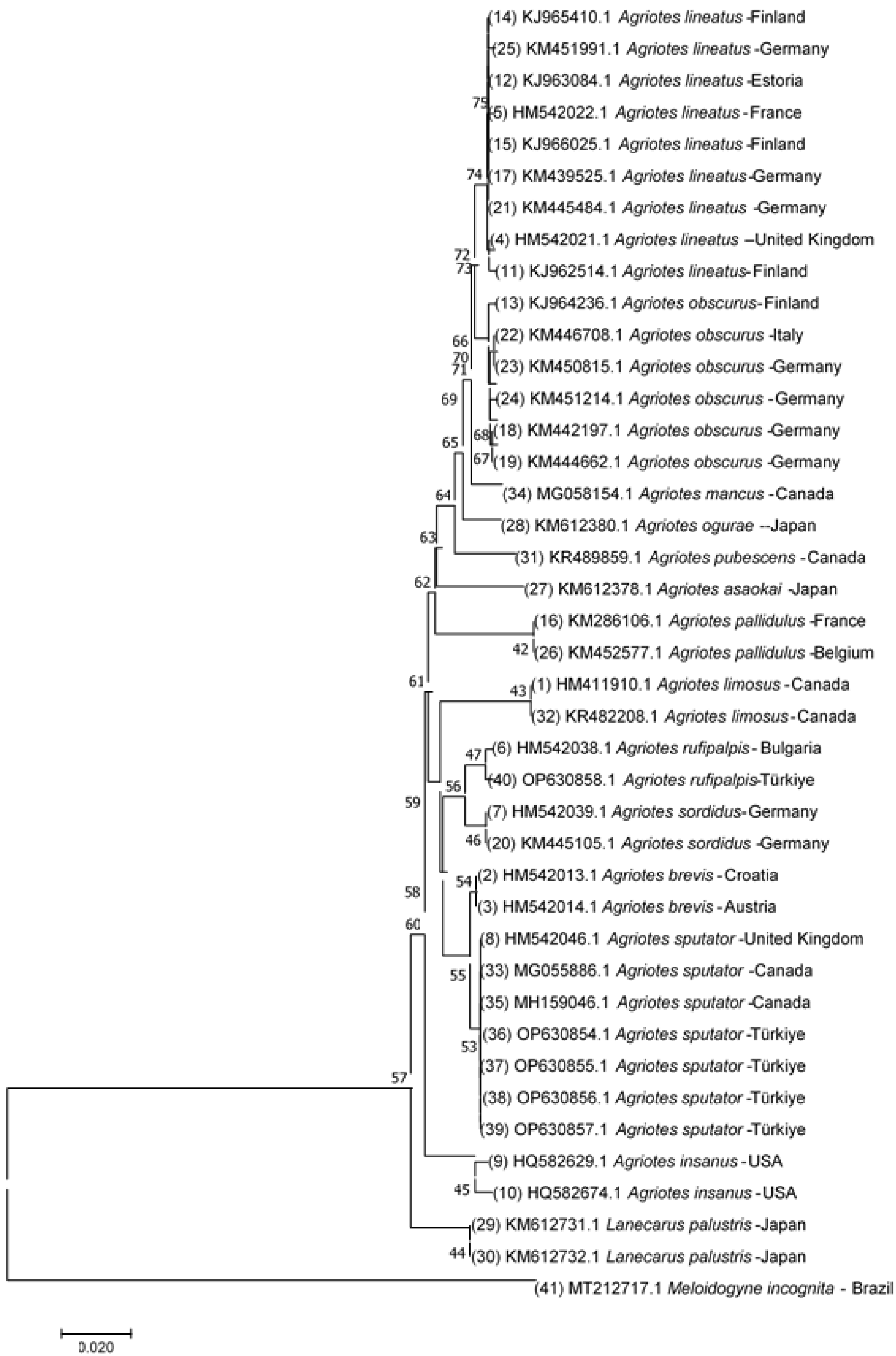

Figure 2. Phylogeny of *Agriotes* specimens generated from Maximum Likelihood analysis of mitochondrial COI sequences, rooted with *Meloidogyne incognita* (Kofoid & White, 1919). Bootstrap support (BS) values >75% are given at the internodes.

Phylogenetic tree shows two distinct clades for the distribution of two Turkish wireworm species (*Agriotes sputator* and *Agriotes rufipalpis*) (Figure 2). Sequencing data confirmed that COI gene sequences showed 100% identity for the populations of *A. sputator,* which were isolated from different areas of Türkiye. The population of A*. sputator* falls under the subclade sharing similarities with the wireworm species found in Canada and United Kingdom. While *A. rufipalpis* species falls under the same subclade of Bulgarian wireworm species. The sequences were deposited in GenBank with the accession numbers for *A. sputator* OP630857.1, OP630856.1, OP630855.1, OP630854.1 and *A. rufipalpis* OP630858.1.

To our knowledge, this is the first study that reports the extensive field survey of wireworm species in the major potato-growing areas of Türkiye. Two wireworm species were detected using molecular methods based on the DNA sequences of COI locus in surveyed locations. *Agriotes sputator* was more common than *A. rufipalpis* and determined in 4 out of 7 surveyed provinces. Similar to our results, *A. sputator* was detected as one of the most widespread species in Türkiye in earlier studies (Kabalak, 2018). Kabalak & Sert (2021) found that *A. sputator* was the most encountered species among 53 wireworm species, with 70 specimens in the Eastern Black Sea Region of Türkiye. In another study, *A. sputator* was one of the abundant species in the Central Anatolian Region of Türkiye (Kabalak & Sert, 2011). Cate (2007) also reported that two wireworm species, *A. lineatus* and *A. sputator*, were the most prevalent species in Türkiye. However, these studies found no specimens belonging to *A. rufipalpis*. Earlier studies suggest 483 described Elaterid species in different parts of Türkiye (Kabalak, 2018). However, only two species were found in potato-growing areas in the current study. Low species diversity found in this study might be due to sampling habitats, collecting methods, seasonal fluctuation of population densities of wireworms, and horizontal and vertical distribution of wireworm species in soil depending on the climatic factors (Kuhar & Alvarez, 2008; Milosavljević et al., 2017). Earlier studies revealed that the feeding activity of wireworms in fields might vary across species according to the types of crops. Cherry (2007) and Kuhar & Alvarez (2008) reported that *Melanotus communis* (Gyllenhal, 1817) (Coleoptera: Elateridae) and *Conoderus* spp. were found less active in the summer months due to unfavorable conditions. Previous studies were conducted at different habitats, including forest, herbaceous plants, and bushes, with varying collection methods such as light traps and insect nets (Kabalak et al., 2013; Kabalak, 2018; Kabalak & Sert, 2021). However, sampling was made by only digging out the soil in this study, and *A. sputator* was the dominant wireworm species in sampling areas. The sample collection time is another factor affecting the acquisition of the wireworm specimens. Previous studies showed that wireworm species are highly active in May and June, which agrees with our sampling time (Kabalak & Sert, 2011; Kabalak, 2018). The low species diversity found in this study may also be attributable to the ability of wireworm species to tolerate seed-applied insecticides. The interviews with the landowners showed that the sampling sites of wireworms were treated with various seed-applied insecticides at least once, which may have affected the species diversity in the sampled fields.

Providing an estimate of species diversity within a community (Shannon, 1948), the Shannon biodiversity index of wireworms was low in potato growing areas in this study (Table 3). This means that in potato fields, wireworm species may be dominant over other species, or that only a few species may possibly have effects on the wireworm community or only a few species may prefer a selective host (potato). This suggests that it may be related to monoculture agriculture in potato growing areas.

Agriotes rufipalpis is a wireworm species known to occur in Türkiye and identified only in Afyon province in this study. Wireworm species have different ecological requirements, such as temperature, soil characteristics, and precipitations (Staudacher et al., 2013). Previous wireworm surveys demonstrated that *A. ustulatus* occurred at warmer and drier climates while *A. brevis* favors higher temperatures and less precipitation (Furlan, 1998; Furlan & Toth, 2007; Lindroth & Clark, 2009; Staudacher et al., 2013). In this regard, our results indicate that *A. rufipalpis* might have found themselves wide range of distribution areas in Afyon.

Conclusions

Morphological identification of wireworm species is quite compelling due to the lack of clear distinguishing characters among highly similar wireworm species. Misidentification of wireworm species might lead to inappropriate management strategies. The results indicated that two wireworm species (*A. sputator* and *A. rufipalpis*) were present in the surveyed potato growing areas and *A. sputator* was the dominant wireworm species in sampling areas. The present study validated the COI region's barcoding significance by successfully discriminating two species in this study from each other and other *Agriotes* species included in phylogenetic analyses.

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