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ORIGINAL ARTICLE

New alien and invasive bamboo aphid species of the genus *Takecallis* (Hemiptera: Aphididae) recorded in Poland – morphological and molecular identity

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Abstract

The occurrence of three bamboo aphid species of the genus *Takecallis* was detected. *T. arundicolens* (Clarke) and *T. arundinariae* (Essig) were recorded for the first time in Poland, and new localities for *T. nigroantennatus* Wieczorek were found. Key diagnostic morphological characteristics to help distinguish these species and DNA barcoding to analyze individuals at the molecular level were provided.

Keywords: bamboo, COI, pest, *Takecallis arundinariae*, *Takecallis arundicolens, Takecallis nigroantennatus*

Introduction

Aphids (Hemiptera: Aphididae) are phytophagous insects, with some species considered as serious pests of cultivated, ornamental, forest and wild plants. A large number of generations during the growing season, high female fecundity and parthenogenetic reproduction contribute to rapid colonization and spread of aphids to their host plants. Additionally, in recent years due to global warming there has been an increase in the number of aphid generations over the year and a longer period of colonization on host plants, which makes these insects troublesome (Blackman and Eastop 2000; Skendžić *et al.* 2021).

Biological invasion is one of the main causes of global biodiversity loss and global ecosystem function erosion. At the same time, it also has a negative impact on human life and health, agriculture, and food security (Ricciardi *et al.* 2021). Among aphids there are numerous species which are treated as serious pests of economic importance. A separate category includes species of foreign origin, which often have an invasive status and are increasing significantly in numbers in Poland. In the 19th century there were three species, and in the 20th century 25 more species of non-native origin were found in Poland. In the 21st century 19 alien species of aphids have been recorded, which shows that in the last two decades several such species of aphids were identified in our country almost every year. So far, 47 species of foreign aphids have been found in Poland, which accounts for almost half of all previously identified species of foreign aphids in Europe (Wieczorek 2011, 2022). In 2023 the alien species of oriental origin – *Takecallis nigroantennatus* Wieczorek was described and recorded for the first time in Poland. This cryptic species, associated with the cold-hardy bamboo variety *Fargesia* spp. (Bambusoideae), was known from one locality in the

Central Pomerania region (Wieczorek and Sawka-Gądek 2023). At the same time, it was the first representative of the genus *Takecallis* Matsumura, detected in Poland (Wieczorek 2023).

Since there are dozens of varieties of cold-hardy bamboo which are popular for gardening and can tolerate snowy winters and freezing temperatures, it was interesting to determine whether other species of the *Takecallis* genus known in Europe, also occur in Poland. Thus, in this paper we address the following questions: (1) how many species of the genus *Takecallis* found in Europe also occur in Poland; (2) if they exist, what bamboo species are they associated with; (3) can these species be easily determined macro and microscopically; (4) if the use of standard barcoding will allow for quick identification of these species, including cryptic ones.

Materials and Methods

Specimen collection and identification

The aphids (adult winged viviparous females) were collected directly from the host plants using a fine brush and placed into Eppendorf tubes containing 70 or 98% ethanol. Totally, the fresh material contained at least 40 individuals from each locality and species and this material was observed and photographed using a Nikon SMZ 25 stereoscopic microscope with a DSFi2 camera. Fourteen individuals from each locality and species were slide-mounted using the method of Wieczorek 2020 in Wieczorek and Chłond (2020), and examined using a Nikon Ni-U light microscope equipped with a phase contrast system. Field photographs were taken using an iPhone 7 camera with the OlloClip Macro Pro Lens Set and Realme GT 2 Pro with Macro Zoom 40x.

Samples were identified based on their morphological diagnostic features using specific literature-based keys (Blackman and Eastop 2023; Wieczorek and Sawka-Gądek 2023). Voucher specimens were deposited in the entomological collection of the University of Silesia, Katowice, Poland (DZUS). The botanical nomenclature was taken from the International Plant Names Index. The map was from Free Vector Maps.com. The figures were prepared using Corel Draw 2021, Corel Corporation.

DNA extraction, PCR amplification, and sequencing

DNA was extracted from ethanol-fixed single individuals using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. In total, 13 specimens were used (seven for *T. arundinariae*, four for *T. arundicolens*, and two for *T. nigroantennatus*). To characterize the molecular taxonomic identity of the specimens and confirm morphological assignment, we amplified the fragment of mitochondrial cytochrome c oxidase subunit I (COI) with the following two "universal" primers: LCO1490 and HCO2198 (Folmer *et al.* 1994). Polymerase chain reactions (PCRs) were carried out in 50 µl reactions consisting of 20 µl ddH2O, 25 µl of Color OptiTaq PCR Master Mix (2x) (EURx, Gdansk, Poland), 1 µl of each primer at 10 mM concentration, and 3 µl of total genomic DNA. Thermal conditions for the reactions were: 1 cycle at 95°C for 180 sec (initial denaturation), and the next 35 cycles, each at 95°C for 30 sec, 45°C for 30 sec and 72°C for 45 sec, with a final 72°C extension for 120 sec. To analyze amplification products, samples were run on a 1.0% agarose gel in TBE buffer with the addition of SimplySafe (EURx). Amplification products were sent to GenoMed (Warsaw, Poland) and sequenced in both directions with the same primers as for PCR.

Phylogenetic analysis

A Maximum Likelihood (ML) analysis was meticulously conducted to infer phylogenetic relationships among the studied taxa, leveraging the sequence information derived from the COI. Comparing with the neighbor-joining (NJ) method, the ML analysis involved the optimization of parameters such as substitution rates, base frequencies, and branch lengths under a carefully selected model of sequence evolution, ensuring a robust and accurate reconstruction of the evolutionary tree for comprehensive insights into the genetic relationships within and between the investigated organisms, including aphids (e.g. Lee *et al.* 2017; Podmore *et al.* 2019; Li *et al.* 2021; Wieczorek and Sawka-Gądek, 2023). The dataset of COI haplotypes from *Takecallis* specimens analyzed in this study was expanded by including sequences used in a previous study by Wieczorek and Sawka-Gądek (2023). Nucleotide sequences were aligned in MEGA7 (Kumar *et al.* 2016) and trimmed if they exceeded the 'Folmer region' (excluding primer sequences). The final COI matrix used for phylogenetic analysis was comprised of 658 sites covering the 'Folmer region' and included 108 terminals. The IQ-TREE web server (Trifinopoulos *et al.* 2016) was used to estimate the best models for nucleotide evolution for the alignment, and, subsequently, to construct a maximum likelihood phylogenetic tree. The tree was constructed with 1000 replications, and bootstrap support values were calculated with 1000 replicates using the ultrafast algorithm with default settings. In addition, the SH-aLRT branch test was used to evaluate the tree branch supports. The tree was rooted at *Calaphis magnoliae* Essig & Kuwana and

Euceraphis lineata Baker, and visualized with iTOL (Letunic and Bork 2007). For visual clarity, most duplicated sequences originating from different specimens were hidden in the final tree.

Results

Morphology

During a field study conducted from May to October 2023 in two regions of Poland (Wielkopolskie and Śląskie Voivodeships), three species of *Takecallis* viz., *T. arundicolens* (Clarke, 1903), *T. arundinariae* (Essig, 1917) and *T. nigroantennatus* Wieczorek, 2023, were found. *T. arundicolens* and *T. arundinariae* were recorded for the first time in Poland, whereas new localities for *T. nigroantennatus* were found. Although the three species mentioned may occur on the same host plant (sometimes in mixed colonies), identification of the dominant morph – winged viviparous female, at both the macro and microscopic level, is possible.

Winged viviparous females of *T. arundinariae* are whitish, pale yellow or grayish-yellow, with variegated antennae, longitudinal dark stripes on the thorax and paired black elongate spots on abdominal tergite 1–7 (Fig. 1A). Both *T. arundicolens* and *T. nigroantennatus* are yellow, however *T. arundicolens* is paler, with variegated antennae and black cauda (Fig. 1B), whereas *T. nigroantennatus* is uniformly dark yellow with black antennae, except for the antennal segments I–II, and the very base of segment III, which are pale (Fig. 1C). These features are clearly visible in live and fresh fixed individuals (Fig. 1D–F). Slide-mounted specimens of all species are transparent, but features such as the colors of the antennae, cauda, and the presence or absence of markings on the thorax and abdomen allow for the correct identification of species of the *Takecallis* genus found in Poland. Additional morphometric features, facilitating the identification of the

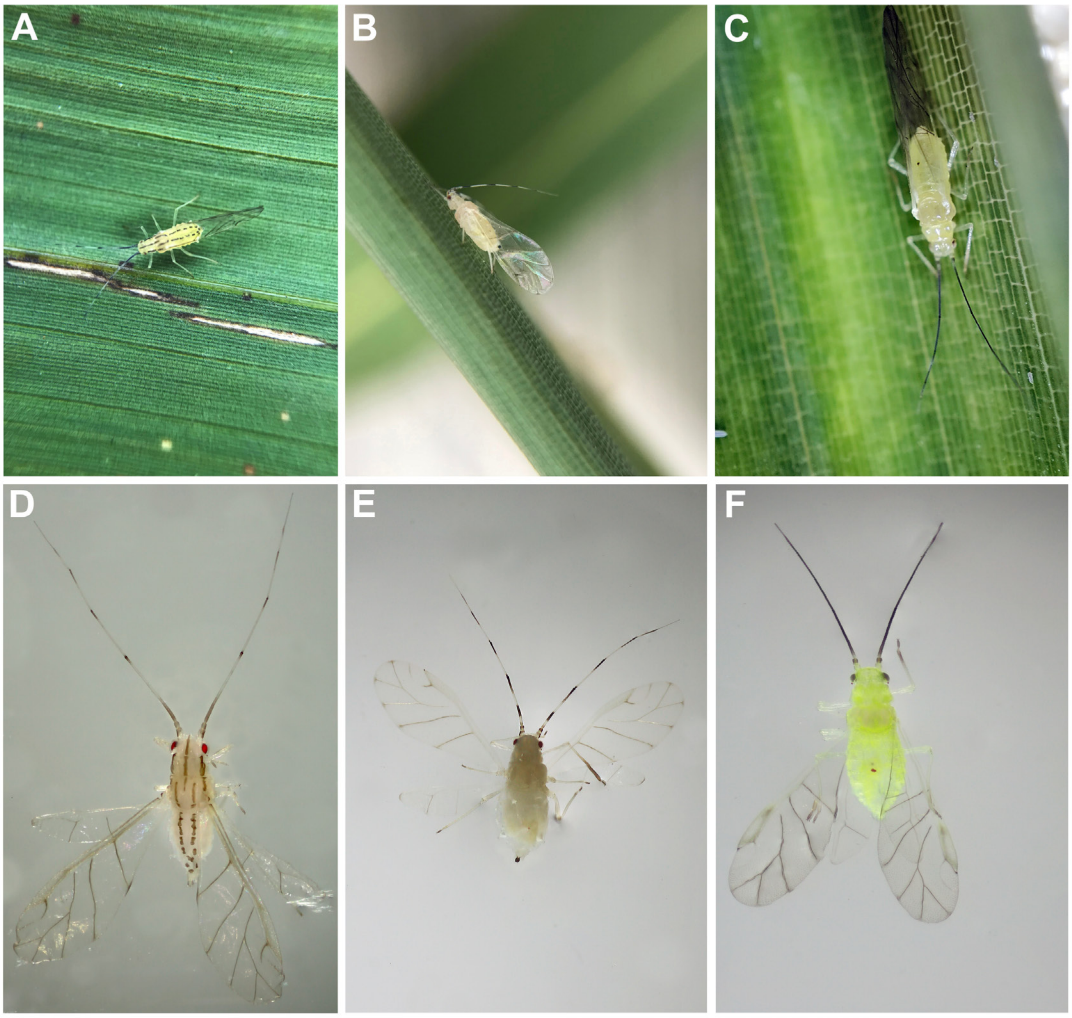

Fig. 1. Living (A–C), fixed in 70% ethanol and observed in the light microscopy (D–F) specimens of winged viviparous females of *Takecallis arundinariae* (A, D), *T. arundicolens* (B, E) and *T. nigroantennatus* (C, F) recorded in Poland

above-mentioned species, are presented in the key to the identification of aphids of the genus *Takecallis*, detected in Poland so far.

Key to the identification of aphids of the genus Takecallis, detected in Poland:

1. Thorax and abdomen without dark dorsal markings, never with paired elongate dark abdominal patches. Pale or blackish cauda...2
– Thorax with variably-developed longitudinal dark stripes, and abdomen with a pair of elongate dark patches on each tergite. Pale caud..........*T. arundinariae*
2. Black antennae, except for antennal segments I-II, and the very base of segment III, which are pale, 1.57–1.90 mm long. Pale cauda ….... *T. nigroantennatus*
– Antennae variegated, 2.36–2.51 mm long. Black cauda ....*T. arundicolens*

Molecular analyses

The 658 bp DNA fragment of the COI sequence obtained for *T. nigroantennatus* TNIGRO4 was identical to that of *T. nigroantennatus* isolate 1BAA (GenBank Acc. OR001758). Similarly, the COI sequence for *T. arundicolens* TARUC3 matched with *T. arundicolens* isolate CZ9-1 (GenBank Acc. KY307022). The COI sequence for *T. arundinariae* TARU1 was also found to be identical to *T. arundinariae* isolate S17 (GenBank Acc. KY307054). However, the newly generated sequence for *T. arundinariae* TARU2 showed a slight variation, sharing 99.85% identity with *T. arundinariae* isolate S17 (GenBank Acc. KY307054). The sequence for this new COI haplotype was deposited in the GenBank database under accession number OR889349. Even though the difference of 0.2% for this group of aphids is very low, due to some unresolved

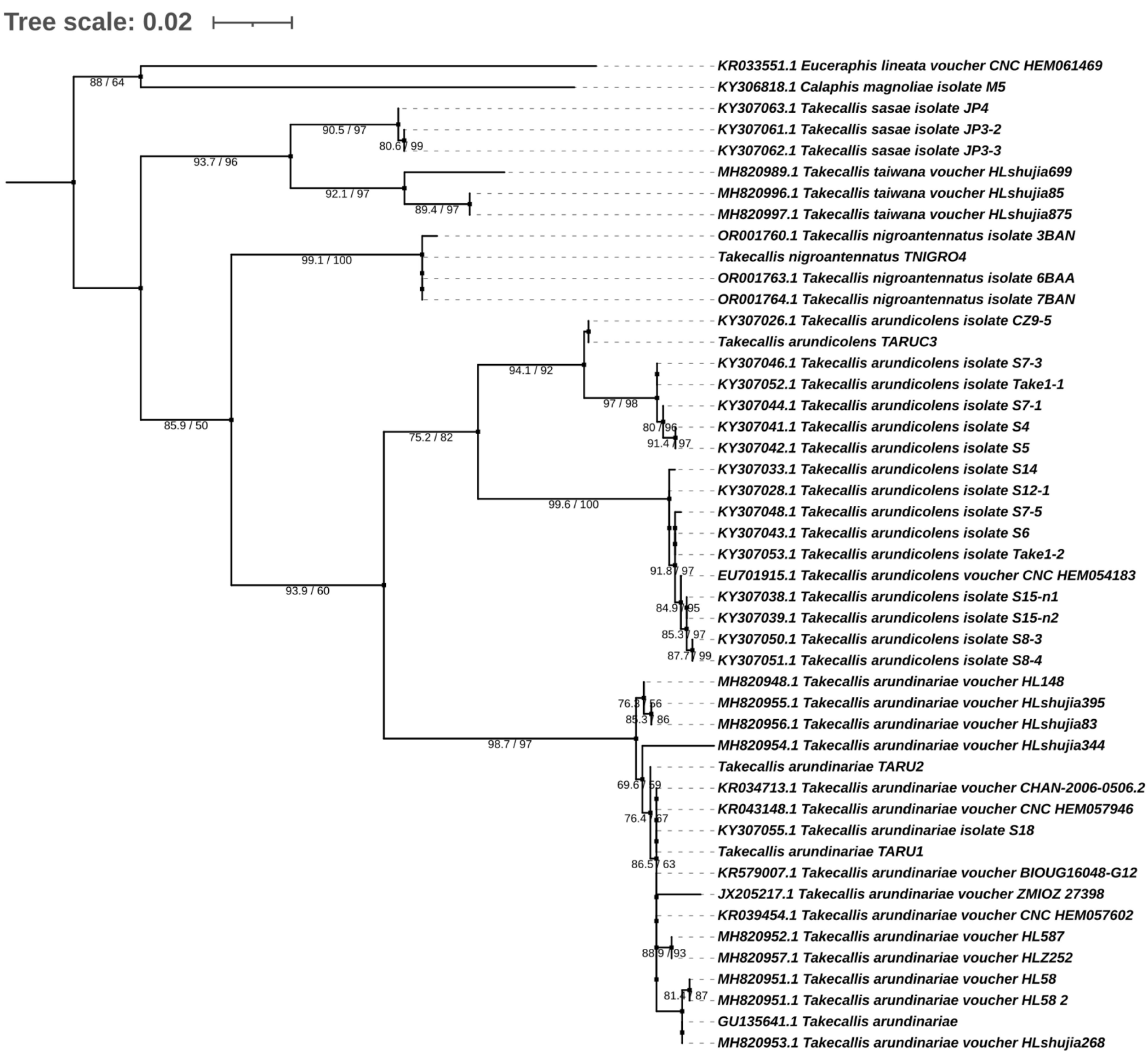

Fig. 2. Maximum likelihood tree resulting from the analysis of COI nucleotide sequences of *Takecallis* specimens with W-IQ-TREE SH-aLRT and UFBoot support values were given below the respective branches. The tree was rooted at *Calaphis magnoliae* and *Euceraphis lineata*

Table 1. Uncorrected pairwise genetic distances (p-dist.) for COI calculated between investigated isolates of *Takecallis* spp.

	Seq. No.	1	2	3	4
T. nigroantennatus TNIGRO4	1		0.011	0.012	0.012
T. arundicolens TARUC3	2	0.088		0.011	0.011
T. arundinariae TARU2	3	0.096	0.081		0.002
T. arundinariae TARU1	4	0.098	0.079	0.002	

taxonomical problems within the genus *Takecallis* (e.g. Lee *et al.* 2017) and further molecular phylogenetic study, we included this result. The uncorrected pairwise p-distances between investigated isolates of *Takecallis* spp. in this study were shown in Table 1. The number of base differences per site from between sequences was shown. Standard error estimates are shown above the diagonal. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 658 positions in the final dataset. Analysis was conducted in MEGA7.

Furthermore, Maximum Likelihood (ML) analysis (Fig. 2) supported each of the three *Takecallis* species examined in this study as distinct species based

Fig. 3. Host plants of the bamboo aphid of the genus *Takecallis* recorded in Poland: A – *Fargesia nitida*, the host plant of *T. arundinariae* in the Japanese Garden of the Botanic Garden in Zabrze; B – *Phyllostachys bissetii,* the host plant of *T. arundinariae* in a private garden in Przeźmierowo; C – *Phyllostachys aureosulcata* 'Spectabilis', the host plant of *T. arundicolens* in the Jasin garden center; D – *Phyllostachys* spp., the host plant of *T. nigroantennatus* in the Tarnowo Podgórne garden center; E – *Fargesia murielae,* the host plant of *T. nigroantennatus* in the Botanic Garden AMU in Poznań

on DNA sequence data, which is in congruence with recent species delimitation by Wieczorek and Sawka--Gądek (2023).

Host plants and localities in Poland

Takecallis arundinariae was recorded for the first time in two localities in Poland. In the Japanese Garden of the Botanic Garden in Zabrze, Śląskie Voivodeship (50.19N 18.47E), associated with *Fargesia nitida* (Mitf.) P.C.Keng 'Eisenach' (Fig. 3A) and in a private garden in Przeźmierowo (52.43N 16.76E), Wielkopolskie Voivodeship, on *Fargesia rufa* T.P.Yi and *Phyllostachys bissetii* McClure (Fig. 3B). In the Botanic Garden in Zabrze, the *Fargesia* on which aphids of the *Takecallis* genus were found, had been cultivated in the Japanese Garden for about 15 years. In Przeźmierowo the mentioned bamboo species had been cultivated for two years, and the plants came from the Netherlands.

Takecallis arundicolens was also found for the first time in two localities in Poland: in the garden centers in Tarnowo Podgórne (52.45N 16.64E) and in Jasin (52.40N 17.12E), Wielkopolskie Voivodeship. They were found on various host plants: *Phyllostachys nigra* (Lodd. ex Lindl) Munroe, *P. aureosulcata* McClure 'Spectabilis', *P. bissetii* and *Pseudosasa japonica* (Siebold & Zucc. ex Steud.) Makino ex Nakai (Fig. 3C). The plants had been imported from Italy.

Takecallis nigroantennatus was found in two localities. In a garden center in Tarnowo Podgórne it was detected in mix colonies with *T. arundicolens* on *Phyllostachys* spp. In the Botanic Garden AMU in Poznań (52.25N, 16.53E), Wielkopolskie Voivodeship, this aphid species was associated with *Fargesia murielae* (Gamble) Yi, growing in the garden area since 1996 (Fig. 3D). Localities of the bamboo aphid of the genus *Takecallis* recorded in Poland are presented in Figure 4.

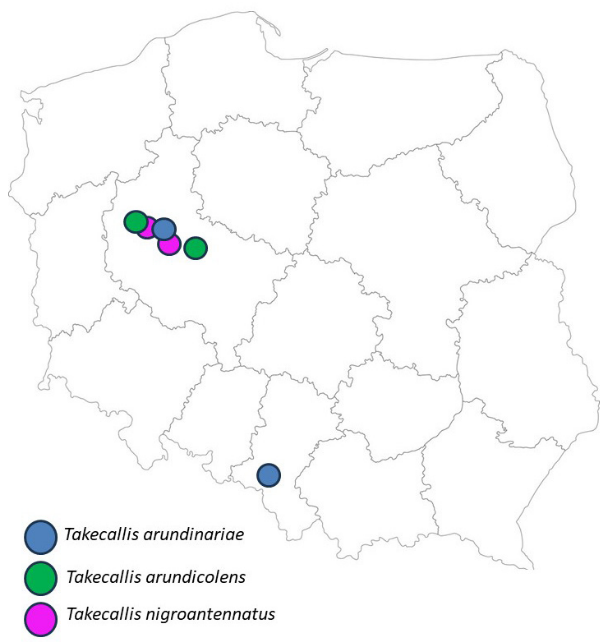

Fig. 4. Localities of the bamboo aphid of the genus *Takecallis* recorded in Poland

Material examined

SA02-367-02-007 *Takecallis arundinariae*, two alate viviparous females, Zabrze Botanic Garden, Poland, 29.V.2023, *Fargesia nitida*, K. Wieczorek leg.; SA02-367-02-008 *Takecallis arundinariae*, two alate viviparous females, Zabrze Botanic Garden, Poland, 27.IX.2023, *Fargesia nitida*, K. Wieczorek leg.; SA02-367-02-009 *Takecallis arundinariae*, two alate viviparous females, Przeźmierowo, Poland, 15.VII.2023, *Fargesia rufa*, B. Borowiak-Sobkowiak leg.; SA02--367-01-003 *Takecallis arundicolens*, two alate viviparous females, Tarnowo Podgórne, Poland, 10.IX.2023, *Phyllostachys bissetii*, B. Borowiak-Sobkowiak leg.; SA02-367-01-004 *Takecallis arundicolens*, two alate viviparous females, Jasin, Poland, 18.IX.2023, *Pseudosasa japonica*, B. Borowiak-Sobkowiak leg.; SA02--367-04-001 *Takecallis nigroantennatus*, two alate viviparous females, Tarnowo Podgórne, Poland, 18.IX.2023, *Phyllostachys nigra*, B. Borowiak-Sobkowiak leg.; SA02-367-04-002 *Takecallis nigroantennatus*, two alate viviparous females, Botanic Garden AMU, Poznań, Poland, 24.X. 2023, *Fargesia murielae*, B. Borowiak-Sobkowiak leg.

Discussion

Aphids are a large and diverse group of insects originating from temperate regions of the Northern Hemisphere. Some species are harmful pests on cultivated, ornamental, forest and wild plants, also capable to transmitting plant viruses (Dedryver *et al.* 2010). More than 100 alien aphid species have been reported in Europe (Couer d'Acier *et al*. 2010; Wieczorek 2022). However, this number is continuously changing due to the increasing globalization of trade in plants and plant material, together with climate change. Consequently, it leads to an increase in the introduction and spread of new and damaging plant pests and pathogens causing serious losses in plant production.

Bamboos were not cultivated in western European gardens until the early 19th century or possibly the late 18th century. Tropical species of the genus *Bambusa* spp. (suitable for greenhouses) from India were the first to be introduced to Europe, followed by temperate bamboo genera *Phyllostachys* spp. and *Pseudosasa* spp. from Japan or China in the mid-19th century. Since the first introduction of bamboos, about 400 different genotypes have been imported to Europe. In particular, in the 1980s and 1990s cold resistant bamboos genera *Fargesia* spp. or *Borinda* spp. were introduced from China. Some fast-growing perennials of the subfamily Bambusoideae (e.g. *Pseudosasa japonica*) had become quite common by then, and are now considered part of the naturalized flora of many European countries (Stapleton 2023). Along with exotic plants, the insects associated with them were also introduced inadvertently. So far, four species of aphids of the *Takecallis* genus have been found in Europe. *T. arundicolens* and *T. taiwana* (Takahashi, 1926) were first recorded in the United Kingdom in 1923, whereas *T. arundinariae* in 1961, also in the UK (Laing 1923; Couer d'Acier *et al.* 2010). Recently described *T. nigroantennatus* was recorded in Poland (Wieczorek and Sawka-Gądek 2023), however its presence (as an unidentified species) was already mentioned in 2015 in the UK (Stapleton 2023), and was confirmed in Belgium (Wieczorek and Sawka-Gądek 2023). All of them are treated as a pest on Bambusoideae and have been considered as invasive organisms in different parts of the world where bamboos are purposefully or accidentally introduced (Rakshani *et al.* 2017).

Bamboos are one of the most economically important plant groups globally, but world-wide trade creates risks of invasions (Canavan *et al.* 2017), on both plants (mostly from the genera *Bambusa* and *Phyllostachys)* and insects associated with them (e.g. *Takecallis* genus). Studies on the history of many invasions have shown that species experienced a short stay in their initial invasion sites before forming a large invasion threat, eventually producing a bridgehead effect, where a colonizing population became the source of multiple new invasive populations (Lombaert *et al.* 2010; Lesieur *et al.* 2019) or an over bottleneck effect, where invading populations with low genetic potential and low reproductive fitness can still become successful in their new environment (Zepeda-Paulo *et al.* 2016; Li *et al*. 2023). Our observations clearly indicate that in the case of aphids of the genus *Takecallis* we are dealing with the bridgehead effect. In botanic gardens, due to the availability of host plants, the populations of the studied species are probably established. At the same time, garden centers seem to be the source of the spread of bamboo aphid species. A large accumulation of seedlings of various species in one place, even with the introduction of single individuals of aphids, causes them to quickly colonize neighboring plants – the "hop on, hop off" effect (Wieczorek and Chłond 2020). Therefore, with the growing popularity of bamboos as ornamental plants, we can expect both new localities of aphids of the *Takecallis* in Poland (or more broadly in that part of Europe where climatic conditions allow frost-resistant bamboo varieties to survive), as well as the presence of other species of this genus introduced to Europe so far (i.e. *T. taiwana*). Thus, early detection and rapid response (EDRR) is a key tenet of invasive (often non-native) species management, where "detection" is the process of observing and documenting an invasive species, and "response" is the process of reacting to the detection once the organism has been authoritatively identified and response options have been assessed. Early detection, rapid assessment and response is a critical defense against the establishment of invasive populations (Reaser *et al*. 2020). In particular, integrated taxonomy - observations of live specimens of aphids and associated host plants, macro and microscopic observations and, finally, rapid barcoding identification can be an effective tool in recognizing alien, often invasive aphid species. That, with the discovery of the three aphids reported here, led to 50 alien species registered for the Polish territory.

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