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Research note

First report of the giant conifer aphid, *Cinara pinivora* (Wilson) and the Monterey pine aphid, *Essigella californica* (Essig) (Hemiptera: Aphididae) in South Africa

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Pine aphids represent a significant group of insect pests in coniferous ecosystems worldwide. To assess pine aphid prevalence and potentially new introductions in South Africa, aphid samples from various pine-growing regions were collected through national surveys conducted by the Tree Protection Co-operative Programme (TPCP) and directly sent from landowners to the FABI Diagnostic Clinic. Morphological examination and DNA barcoding confirmed two previously unreported aphid species as *Cinara pinivora* and *Essigella californica*. To our knowledge, this is the first report of these North American native aphids in South Africa. This study provides an important reference for future research aimed at safeguarding South Africa's pine plantations against emerging threats from invasive aphid species.

Key words: CO1, invasive species, pest surveillance

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Introduction

Species of the genus *Pinus* play an important role in South Africa's economy by contributing through the trade of solid timber, paper and pulp (Morris 2022; van Wilgen and Richardson 2012). Pines occupy approximately 48.3% of the country's 1.2 million hectares allocated for forestry activities (Morris 2022). Species cultivated since 2010 include *Pinus elliottii*, *P. patula, P. radiata*, *P. taeda* and their hybrids, *P. patula* × *P. tecunumanii* using high elevation origin provenances of *P. tecunumanii* (PPTH), *P. patula* × *P. tecunumanii* using low elevation origin provenances of *P. tecunumanii* (PPTL) and *P. elliottii* × *P. caribaea* var. *hondurensis* (PECH) (Morris 2022). Despite successful adaptation of pines in South Africa, their production is threatened by native and non-native insect pests (Wingfield et al. 2020). The current insect pests affecting pine trees in South Africa can be categorised into four main groups: defoliators, wood and bark borers, sap-suckers, and pests impacting establishment (Roux et al. 2012). Within the sap-suckers group, non-native aphids (Hemiptera: Aphididae) are of great concern.

In the 1970s, two aphid species were reported as pests on *Pinus* species in South Africa. The black pine aphid, *Cinara cronartii* Tissot & Pepper native to North America (Tissot and Pepper 1967) was reported in 1974 (van Rensburg 1979). Subsequently, the pine needle aphid, *Eulachnus rileyi* (Williams), native to Europe was reported in 1978 (Blackman and Eastop 1994; Bruzas 1981; Marchant 1981). *Cinara cronartii* colonies thrive on pine tree branches, especially near growing tips with thin bark. Severe infestations can stunt shoot growth and cause die-off of tops of pines and branches, potentially leading to tree death in stressed conditions (van Rensburg 1979). *Eulachnus rileyi* feed on the underside of pine needles at the tree crown, turning needles yellow and stunting tree growth during infestations (Chilima 1991; Murphy et al. 1991). Stressed trees may experience top die-back or even death. In 1978, Zwolinski (1989) reported the presence of the pine woolly aphid, *Pineus pini* (Macquart), which is in fact an adelgid. *Pineus pini* damage can result in dying branches, tree mortality, foliage discoloration, distortion, reduced plant stand growth, and resinosis (Mailu et al. 1978; McClure 1982).

The rate of introduction of non-native forest species has increased drastically over the last few decades, with some of the introduced species resulting in substantial damage (Hurley et al. 2016, 2017; Wingfield et al. 2015). Thus, the emergence of new non-native pests, including aphid species,

is a significant concern, necessitating continuous monitoring for incursion detection. Recent samples obtained from different pine-growing regions (Figure 1) unveiled two aphid species not previously documented. This study reports on the identification and distribution of the two new invasive aphid species in South Africa.

Materials and methods

Aphid collection and preservation

Pine aphid collections were performed between June 2019 to June 2023. The samples were collected either through national surveys conducted by the Tree Protection Co-operative Program (TPCP) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, or sent directly to the FABI Diagnostic Clinic from landowners in various pine-growing regions across Eastern Cape, Western Cape, KwaZulu-Natal, and Mpumalanga provinces of South Africa. Host records for pine-aphid association were recorded during collections of pine aphid. All collected pine aphid samples were preserved in 70% ethanol and stored at −20 °C until used for molecular analysis.

Identification

Morphology

The tentative identifications of aphid species were determined using the online identification keys given in Blackman and Eastop (2024) under a Nikon SMZ1500 stereoscopic microscope (Nikon, Tokyo, Japan). Thereafter, relevant species original descriptions were checked (Hottes 1959; Sorensen 1994; Wharton et al. 2004). Images of the aphids were taken with an Olympus DP21 camera system connected to a Nikon SMZ1500 microscope to maintain accurate records of the identified aphid species. Body length measurements of aphid species were then processed using Olympus Stream Basic image analysis software.

Molecular

Specimens preserved in 70% ethanol were rinsed in sterile distilled water to remove excess ethanol before DNA extraction. Total genomic DNA extraction from two front legs of individual specimens were performed using a *prep*GEM® Tissue Kit (ZyGEM) following manufacturer's instructions with the following modification. DNA concentration was measured using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, USA) and adjusted to 2 ng μl^{-1}. PCR amplification of the cytochrome oxidase subunit I mitochondrial gene (COI) were performed on extracted DNA using LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994) to yield a 710 bp fragment. The PCR reactions were run on a Bio-Rad iCycler thermocycler (BIO-RAD, Hercules, CA, USA), following the protocol described by Folmer et al. (1994). Thermocycling parameters consisted of denaturation at 95 °C for 1 min, followed by 35 cycles of 95 °C for 45 s, 51 °C for 45 s and 72 °C for 1 min, with a final extension at 72 °C for 3 min. The successful amplifications of the gene region (COI) were confirmed by staining 2 µl of each PCR product with 1 µl of GelRed™ (Biotium, USA) nucleic acid dye. Subsequently, the samples were separated in a 2% SB (1x) buffered agarose mini-gel at 100 V for 40 min, alongside a molecular weight marker (100 bp Ladder, Invitrogen). The resulting products were visualised using a Bio-Rad Gel Doc™ EZ Imager and the Image Lab v4.0 build 16 software.

The PCR products underwent purification through ExoSAP-IT treatment (USB Corporation, Cleveland, OH). In this process, 8 µl of ExoSAP-IT was added to each PCR product and incubated at 37°C and 80°C for 15 minutes at each temperature, followed by a final incubation at 4 °C. For sequencing reactions, both forward and reverse reactions were conducted in a 12 µl reaction volume. This mixture comprised 7.5 µl of ultrapure (SABAX) water, 1.0 µl of sequencing buffer, 1.0 µl of BigDye™ Ready Reaction Mixture with Amplitaq DNA polymerase (Perkin-Elmer Applied Biosystems, Warrington, UK), 0.5 µl of either primer (10 µm), and 2 µl of purified PCR product (50 ng µl-1). The thermocycler conditions involved an initial denaturation step for 2 minutes at 96 °C, followed by 30 cycles at 96 °C for 30 seconds, 54 °C for 15 seconds, and 60 °C for 4 minutes.

Subsequently, the sequencing products underwent purification using the Ethanol/NaAC precipitation protocol from the ABI manual. For this, a mixture of 50 µl of 99% ethanol, 2 µl of sodium acetate (3M), and 8 µl of ultrapure (SABAX) water was added, resulting in a final volume of 72 µl. The mixture was incubated at −20 °C for 10 minutes, centrifuged for 30 minutes at 14 000 rpm, and washed twice with 150 µl of 70% ethanol at 14 000 rpm, with the supernatant being discarded after each run. The pellets of the sequencing PCR amplicons were then vacuum dried and concentrated using an Eppendorf Concentrator 5301 at 60 °C for 20 minutes. Finally, the sequencing of the PCR products was carried out on an ABI PrismTM 3100 Genetic Analyzer (Applied BioSystems, USA).

The DNA sequences were edited using CLC Main Workbench 6.0 (CLC Bio, Denmark) and Biological Sequence Alignment Editor (BioEdit) software (Hall 1999) version 7.0.9. Confirmation of the tentative morphological identity of the pine aphid specimens was obtained by running the COI sequences through the nucleotide Basic Local Alignment Search Tool (BLASTn) against the National Center for Biotechnology Information (NCBI) GenBank database (http://www.ncbi.nlm.nih.gov).

Results

Morphological analysis

In addition to the known aphid species, *C. cronartii* and *E. rileyi*, two morphospecies of aphids were identified based on morphological characteristics. For the first morphospecies, adult body length ranged from 3.5 to 4.1 mm. The wingless adults (apterae) exhibited a glossy dark brown head, a lighter brown thorax and abdomen with dark dorsal sclerites. Additionally, the aphids had black steep-sided siphuncular cones, and their legs featured pale yellow sections. These aphids exhibited characteristic traits associated with the giant conifer aphid, *Cinara pinivora* (Wilson).

The second morphospecies had adult body lengths ranging from 1.5 to 1.82 mm. The wingless adults (apterae) had a spindle-shaped body, very short 5-segmented antennae, a grey-green thorax, and a pale lime green

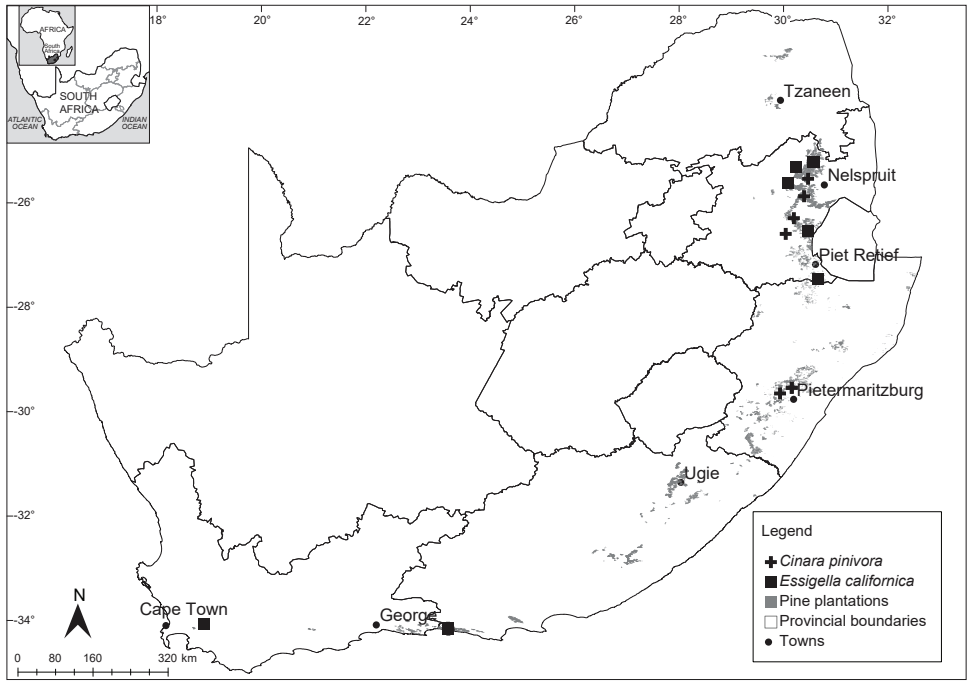

Figure 1: Map of South Africa showing areas from which samples of *Cinara pinivora* and *Essigella californica* were sourced for this study

abdomen, occasionally marked with faint brown dorsal spots. The legs of the aphids showed variable pigmentation, often predominantly pale but with darker tibiae. These aphids exhibited distinctive characteristics indicative of the Monterey pine aphid, *Essigella californica* (Essig).

Molecular analysis

The pine-feeding aphids' consensus sequences were subjected to a BLASTn search to identify matching sequences. The COI sequence of the morphospecies tentatively identified morphologically as *C. pinivora* precisely matched sequences CNJAD1865-12 and GMHKB332-15, both attributed to *C. pinivora,* with a 100% identity. The COI sequence of the morphospecies tentatively identified as *E. californica* matched with sequences GBMIN69109-17, RFBAE937-09, GBMHH27700-19, GMOWJ097-15, and GBMIN69110-17 from the Barcode of Life Data Systems (BOLD) database (https://v3.boldsystems.org), all indicative of *E. californica*, with 100% identity.

Distribution

Cinara pinivora and *E. californica* were confirmed to be present in three and four provinces, respectively (Figure 1; Supplementary Table 1). Both aphid species were collected from similar *Pinus* species, namely *Pinus elliotii*, *P. taeda*, *P. radiata*, and a hybrid species, *P. patula* × *tecunumanii*.

Discussion

Our study reports on the identification of aphids obtained from samples from different pine-growing regions in South Africa. Using a combination of morphological characteristics and DNA sequence data the identity of the new invasive pine aphids specimens was confirmed as *C. pinivora* and *E. californica*. To our knowledge, this is the first official report detailing the presence of these invasive insects in South Africa.

Cinara pinivora is native to North America (Sorensen 1994; Watson et al. 2008) and its main host trees include *Pinus banksiana*, *P. clausa*, *P. echinata*, *P. elliottii*, *P. glabra*, *P. pungens*, *P. resinosa*, *P. rigida*, *P. serotina*, *P. sylvestris*, *P. taeda* and *P. virginiana* (Voegtlin and Bridges 1988). It has been reported invasive in several countries, including Kenya (Mwangi et al. n.d.), Malawi on *P. elliottii* and *P. taeda* (Chilima 2004), Brazil on *P. caribaea*, *P. elliottii*, *P. pinaster* and *P. taeda*. (Iede et al. 1988; Lázzari et al. 2004), Australia, Argentina and Uruguay on *P. elliottii* and *P. taeda* (https://www.fao.org/forestry/49397/en/; accessed January 2024). *Cinara pinivora* initiates pine damage through discoloration and premature needle shedding, accompanied by the browning of certain branches. This leads to a diminished photosynthetic surface, causing stunting and impacting the overall form of host trees, ultimately reducing plant growth. In Brazil, Patti and Fox (1981) observed swelling in branches and plant mortality.

Parasitoid species from the genera *Pauesia* and *Xenostigmus* (Hymenoptera: Braconidae) are commonly employed to manage aphids infesting pine plantations. In Brazil, the parasitoid *Xenostigmus bifasciatus* (Ashmead) (Hymenoptera, Braconidae) was introduced from the USA's east coast. Subsequently, this parasitoid was observed in multiple areas affected by the pine aphid, confirming its successful establishment in Brazil (Penteado et al. 2004).

As with *C. pinivora*, *E. californica* is also native to North America (Sorensen 1994), and has been reported invasive in various countries, including France, Spain, Australia, Brazil, and New Zealand (Carver and Kent 2000; Seco Fernández and Mier Durante 1992; Turpeau and Remaudière 1990;

Zonta de Carvalho and Noemberg Lazzari 2000). The invasive range of *E. californica* beyond its native habitat is likely linked to the wide distribution of its native host, particularly *P. radiata*, which is a commercially important exotic softwood timber species (Lewis et al. 1993). *Essigella californica* is not classified as a pest in North America, but it has emerged as a significant pest in Australia on *P. radiata* (Hopmans et al. 2008). The extent of damage caused by *E. californica* can vary from crown discoloration (needle chlorosis) to crown defoliation. This variation is influenced by factors such as the time of year, and stand age and structure (Eyles et al. 2011).

The parasitoid fauna associated with *Essigella* is relatively understudied. However, approximately 48 species from six braconid genera are known to parasitise the Lachninae (Mackauer and Starý 1967), the sub-family to which *E. californica* belongs. Therefore, it is probable that some of these Lachninae parasitoids could also target *E. californica* if they coexist in the same geographic regions (De Barro and Floyd 2000).

The detection of *C. pinivora* and *E. californica* raises questions regarding their introduction into South Africa. The introduction pathway of the two pine aphids into South Africa remains unknown but was likely through infested plant material or travellers (people) as these are two of the main pathways for introduction of homopteran pests of forests (Meurisse et al. 2018). Furthermore, *C. pinivora* and *E. californica* were found in geographically distant provinces of South Africa, indicating that they were likely present in pine-growing areas of South Africa for several years previous to this report. It is possible that *C. pinivora* and *E. californica* were initially mistaken for *C. cronartii* and *E. rileyi,* respectively, due to the general similarity to those species if not examined more closely. This demonstrates the importance of regular national pest and pathogen surveillance, including the use of appropriate tools to confirm species identification (Hurley et al. 2017).

Conclusion

The confirmation of *C. pinivora* and *E. californica* in South Africa marks the first occurrence of invasive aphids in the country in four decades since the last reported case. At this stage the economic significance of these aphid species, and whether management will be required, is not clear. The lack of reports of high aphid infestations in the last several years (BP Hurley, pers. observation, University of Pretoria, 2024) suggests that these aphids are not currently important pests of pine in South Africa. However, pest status can change over time (Pinkard et al. 2017) and thus it is important that these new aphid species are included in monitoring efforts and awareness programmes. If management is required, the use of biological control, cultural practices and behaviour modifying chemicals are some of the options that could be pursued (Branco et al. 2023).

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