

First report of *Melanterius inconspicuus* (Coleoptera: Curculionidae: Cleogonini) from KwaZulu-Natal, South Africa, with observations of its development in black wattle

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Melanterius is a large genus of small weevils that primarily feed on the seeds of *Acacia* species occurring in their native range in Australia. In the latter part of the 20th century, five seed-feeding *Melanterius* species were released against several weedy *Acacia* species in South Africa as biological control agents. In early 2024, *Melanterius* weevils were detected damaging the vegetative plant tissues of trees in a commercial black wattle (*Acacia mearnsii*) compartment in the KwaZulu-Natal, South Africa. Morphological identification and DNA sequencing revealed that the weevils represent the species *Melanterius inconspicuus*, which is not one of the five species introduced in South Africa. This finding represents the first record of this *Melanterius* species outside of its native range in Australia and infesting black wattle, as well as the first record of a *Melanterius* species infesting vegetative tissues.

Keywords: accidental introduction, invasive species, seed-feeding weevils

Introduction

During the 19th century, several Australian *Acacia* (Fabaceae) species were introduced to South Africa for ornamental, horticultural, or commercial purposes (Richardson and Kluge 2008, van Wilgen et al. 2011, Jansen and Kumschick 2022). However, due to the production of abundant and long-lived seeds and the absence of natural enemies, these trees soon became some of the most invasive weeds in the country, threatening local biodiversity (Dennill et al. 1999; Impson et al. 2000; Richardson and Kluge 2008; Le Maitre et al. 2011; Impson et al. 2021). One such example is *A. longifolia*. Introduced in the late 1820s to help with dune stabilisation, the species is now invasive across the Cape floristic region (Dennill and Donnelly 1991; Impson et al. 2004; van Wilgen et al. 2011). Like other invasive *Acacia* species, it threatens the native fynbos vegetation and many vulnerable species of the region (Impson et al. 2004; Yelenik et al. 2004; Richardson and Kluge 2008; Le Maitre et al. 2011).

In South Africa, introduced *Acacia* species tend to grow in dense thickets and produce large seed banks, making mechanical and chemical control either intensely difficult or completely unfeasible (Donnelly 1992; Richardson and Kluge 2008; Le Maitre et al. 2011). Since the 1970s, options for the biological control of these weeds have been extensively explored. However, this has not come without conflicts of interest between agencies that want to manage the spread of these plants and those that grow them commercially, which is particularly relevant in the case

of *Acacia mearnsii* (black wattle) (Dennill and Donnelly 1991; Impson et al. 2000; Le Maitre et al. 2011; Jansen and Kumschick 2022). Therefore, the selection of suitable biological control agents has largely focused on species that impact the reproductive output of the plants (flowers and seeds), allowing the commercial use of vegetative parts (bark and trunks) to continue (Impson et al. 2009; van Wilgen et al. 2011).

Given these constraints on the selection of control agents, seed-feeding weevils of the genus *Melanterius* Erichson, 1842 (Curculionidae: Cleogonini) have been favoured for their use as biological control agents against invasive *Acacia* species (Donnelly 1992; Impson et al. 2000; Pinzón-Navarro and Oberprieler 2017; Impson and Hoffmann 2019). Currently, 79 species of *Melanterius* are described, which are divided into six species groups: the *M. maculatus* group, the *M. costatus* group, the *M. porcatus* group, the *M. ventralis* group, the *M. laticornis* group and the *M. latipennis* group (Pinzón-Navarro et al. 2017). Apart from *M. servulus*, which also develops in seeds of *Paraserianthes lophantha*, all other *Melanterius* species are only known to develop in the seeds of *Acacia* species (Pinzón-Navarro et al. 2017; Impson and Hoffmann 2019). Due to these favourable characteristics, five *Melanterius* species (i.e. *M. acaciae*, *M. castaneus*, *M. servulus*, *M. maculatus* and *M. ventralis*) have been intentionally introduced to South Africa as biological control agents of ten *Acacia* species and

P. lophantha (Pinzón-Navarro and Oberprieler 2017; Pinzón-Navarro et al. 2017; Impson and Hoffmann 2019).

Melanterius species are univoltine, producing only a single generation of offspring per year. The adults shelter in cracks and crevices in the bark and in old seed pods throughout the year until they reach sexual maturity (Donnelly 1992; Donnelly and Hoffmann 2004). Sexual maturity coincides with flower maturation and seed pod development and commences with mating, after which the females lay single eggs onto a seed inside the developing seed pods (Donnelly 1992). An individual larva spends its entire development feeding on one or, occasionally, a few seeds in the seed pod, and when mature it chews through the wall of the seed pod, falls to the ground and burrows into the soil to pupate. Some larvae pupate immediately and emerge as adults at various times during the following months prior to the next flowering season, but a proportion of larvae remain dormant in the soil and emerge as adults in the following two to three years (Donnelly 1992; Schmidt et al. 1999; Impson and Hoffmann 2019).

In January 2024, during surveillance activities of the National Monitoring of Forest Pest and Pathogen Programme (a government funded initiative to monitor the distribution of forestry pests and diseases throughout South Africa), an unknown weevil was detected infesting young shoots and stems of two-year-old *A. mearnsii* trees near the town of Howick, KwaZulu-Natal. Grown in this area for commercial use in the tannin and timber industries, these trees had been relatively unaffected by wood-boring insects (Hurley et al. 2023). The unexpected occurrence of weevil larvae in the shoots of these trees made their identification a priority, to determine whether they represent a native species or an introduced one with the potential to become a wattle pest in South Africa. A further aim of this study was to present initial observations of the symptoms on the plants caused by the weevil and document some of its behaviour.

Materials and methods

Recovery of weevils and larvae from field sites

Weevils were observed at three different field sites on two different occasions in the KwaZulu-Natal Midlands in 2024. The first observation and collection of the weevils occurred on 29 January 2024 at two sites close to each other in Howick (Site 1 GPS: –29.41841, 30.14415, planted to six-month-old *A. mearnsii*; and Site 2 GPS: –29.42408, 30.14343, planted to two-year-old *A. mearnsii*). During a visit to Site 1, many young trees with yellowing and curling leaves were observed, and on investigation of these symptoms swollen lesions were discovered on the shoots of the trees and the larvae of an unknown weevil were found inside the lesions. In most cases, a hole was clearly visible on the swollen lesions. Upon careful dissection of tissue layers from these lesions, a tunnel containing a larva was found. Some of the larvae were carefully removed using a pair of soft-tipped tweezers and transferred to a vial containing 70% ethanol for transport back to the Forestry and Agricultural Biotechnology Institute (FABI), based at the University of Pretoria. Investigation at Site 2 revealed similar symptoms on the young shoots of the two-year-old trees, and adult specimens of an unknown weevil species were found sheltering in bark crevices and

lesions on the trees. The weevils were also collected in separate vials containing 70% ethanol for transport to FABI.

Morphologically similar weevils were again encountered at Site 2 during a second visit, in August 2024, as well as at a third site located near the town of Richmond, KwaZulu-Natal (Site 3 GPS: –29.7337, 30.29993). Weevils from these two sites were also collected, placed in 70% ethanol and transported to FABI. Additionally, plant material with holes at the site of the swollen lesions was collected from all three sites on both occasions and brought back to FABI for closer examination. The plant material was visually inspected under a microscope, and any adult weevils found were preserved in 70% ethanol. No larvae were found in the vegetative plant material during this subsequent site visit. Vials containing preserved weevils were maintained at –20 °C for further use.

Morphological examination and identification of adult specimens

Initial examination of all collected adult specimens indicated that only one species was present. Three of these specimens were sent to Dr Rolf Oberprieler at the CSIRO Australian National Insect Collection (ANIC) in Canberra, Australia, for identification. Photographs of relevant structural details of the weevils were taken using a Zeiss Discovery V16 dissection microscope with an attached Zeiss Axiocam 512 colour camera (Zeiss, Oberkochen, Germany). A compound photograph of the lateral habitus of the weevil was generated by stacking more than 50 individual image slices using the Helicon Focus v. 5 software (HeliconSoft, Kharkiv, Ukraine).

DNA extraction, sequencing, and phylogenetic analyses

Three adult weevils and one larva were selected for DNA extraction and sequencing. DNA was extracted using the Macherey-Nagel Nucleospin Tissue extraction kit (Separations, Johannesburg, South Africa), following the manufacturer's instructions except that the final elution volume was reduced from 100 µl to 70 µl. DNA was eluted from the column membrane using 35 µl elution buffer, and this step was repeated with a second round of 35 µl elution buffer to bring the total volume to 70 µl. The DNA was stored at –20 °C when not in use.

PCR amplification and DNA sequencing were performed of the partial mitochondrial cytochrome oxidase 1 (COI) region using the primer pair TL2-N-3014 and C1-J-2183 (Simon et al. 1994). PCR was performed using 25 µl reactions containing 2 µl undiluted template DNA, 2.5 µl 10× PCR buffer, 2.5 µl dNTPs (0.5 mM), 1 µl of each primer (10 mM), 0.5 µl MgCl₂ (25 mM) and 0.2 µl KAPA Taq Polymerase (20 units) (Merck KGaA, Darmstadt, Germany). Amplification consisted of an initial denaturation at 95 °C for 15 min, followed by a touchdown cycle consisting of denaturation at 95 °C for 30 s, annealing starting at 55 °C for 30 s and decreasing by 1 °C until reaching 45 °C, and extension at 66 °C for 1 min, followed by 29 cycles of denaturation at 95 °C for 30 s, annealing at 51 °C for 30 s and extension at 66 °C for 1 min, with a final extension at 66 °C for 10 min and a hold at 10 °C.

PCR products were cleaned using ExoSAP-IT™ PCR Product Clean-up Reagent (ThermoFisher Scientific, Massachusetts, United States) to remove residual chemicals. PCR products were sequenced according to the BigDYE® Terminator v3.1 cycle sequencing kit (ThermoFisher Scientific)

protocol in both the forward and reverse directions at an annealing temperature of 55 °C. Sequencing PCR products were precipitated using the sodium-acetate/ethanol method and submitted to the DNA Sanger sequencing facility based at the University of Pretoria, South Africa, for analysis on an ABI PRISM®3500 Genetic Analyzer (Applied Biosystems, California, United States). Quality assessment of sequencing reads was carried out using the software Sequence Scanner 1.0 (<https://sequence-scanner-software.software.informer.com/>), and consensus sequences were generated. Sequences were deposited in NCBI GenBank with accession numbers PQ881942-PQ881945.

The newly generated consensus sequences were used in a BLASTn search of the NCBI sequence database (<https://blast.ncbi.nlm.nih.gov/>) to aid in the confirmation of the morphological identification. A COI dataset was prepared for the genus *Melanterius* using DNA sequences of specimens from ANIC as uploaded to GenBank (Pinzón-Navarro et al. 2017).

Initial preparation of the dataset was done using Mega 7.0.26 (Kumar et al. 2016), after which it was aligned using the online version of MAFFT 7 (Katoh and Standley 2013) with the default settings except for the 'direction of nucleotide sequences' option set to 'adjust direction according to first sequence', so as to ensure that all sequences were in the same orientation. The aligned dataset was assessed and trimmed in Mega 7.0.26 before Maximum Likelihood (ML) analysis using RAXMLGUI 2 (Silvestro and Michalak 2012; Edler et al. 2019). Phylogenetic analyses were performed using ten random initial ML searches followed by 1000 bootstrap replications.

Results

Morphological identification

Identification of the three specimens submitted to ANIC was made firstly by assessing the specimens using the diagnoses and illustrations of the *Melanterius* species published by Pinzón-Navarro et al. (2017) and secondly by detailed comparison with authentically identified voucher specimens of all species of the *M. costatus* group in ANIC. Both methods unequivocally aligned the specimens with *M. inconspicuus*

(Figure 1). Critical matching characters were the paired, oblique ventral carinae between the meso- and metacoxae (shared only with the larger *M. costatus*), the shape of the anterior processes of the mesoventrite between the pro- and mesocoxae and especially the shape and structure of the penis, including the small paired copulatory sclerites in the endophallus (Pinzón-Navarro et al. 2017 Figure 31), which are nearly always species-diagnostic in weevils. An additional likely diagnostic character of *M. inconspicuus* was found in the uncus of the tibiae of the males, being apically bifurcate on the mesotibiae but apically angled on the metatibiae. This character does not occur in *M. costatus* and other species of the *M. costatus* group as examined and appears eminently useful as an additional feature to identify *M. inconspicuus*.

DNA sequencing and phylogenetic analyses

COI sequences were successfully generated from a larva extracted from a tunnel collected at Site 1 in January 2024, an adult weevil collected at Site 2 in January 2024 and two adults collected at Site 3 in August 2024. The four sequences were 100% identical and confirmed that both life stages represent the same species. Maximum-likelihood phylogenetic analyses of these four and of several other COI sequences of *Melanterius* species generated by Pinzón-Navarro et al. (2017) confirmed not only the placement of our specimens in *Melanterius* but also in a clade including two specimens of *M. inconspicuus* sequenced by Pinzón-Navarro et al. (2017) (Figure 2). This clade was well supported and clustered strongly with two other species of the *M. costatus* group, *M. costatus* and *M. tasmaniensis*, separate from the clades representing specimens of the five species introduced into South Africa as biological control agents (Figure 2). The sequences of our specimens differed by eight base pairs from those of both previously sequenced *M. inconspicuus* specimens included in the analyses (ANIC 2412 and ANIC 2631; Pinzón-Navarro et al. 2017), and sequences of these two specimens differed from each other by three base pairs. However, due to the small number of sequences available for the species and the high variability commonly observed in sequences of the COI gene region, this variation is not unexpected.

Initial observations on symptoms and behaviour of *M. inconspicuus* on black wattle

In January 2024, two sites located near each other and planted to six-month-old and two-year-old *Acacia mearnsii* trees at the time, respectively, were visited as part of the surveillance activities of the National Pest and Pathogen Monitoring Programme. Many of the six-month-old trees at the first site were found to be affected by the weevil larvae, suggesting high levels of infestation at this site. Symptoms of infestation on the trees were distinct yellowing and curling of the leaves (Figure 3a) as well as swelling at the branch forks (Figure 3b), which are different from the symptoms caused by other pests or pathogens known to afflict *A. mearnsii* in South Africa. Careful removal of the bark from the swellings revealed the presence of larvae tunnelling and feeding in the shoots (Figure 3c). Although some exit holes were observed on these shoots, no adults were located on the young trees.

Yellowing of young shoots was still noticeable on the two-year-old trees but much less so than on the six-month-old



Figure 1: Lateral habitus of an adult *Melanterius inconspicuus* weevil. Scale 1mm

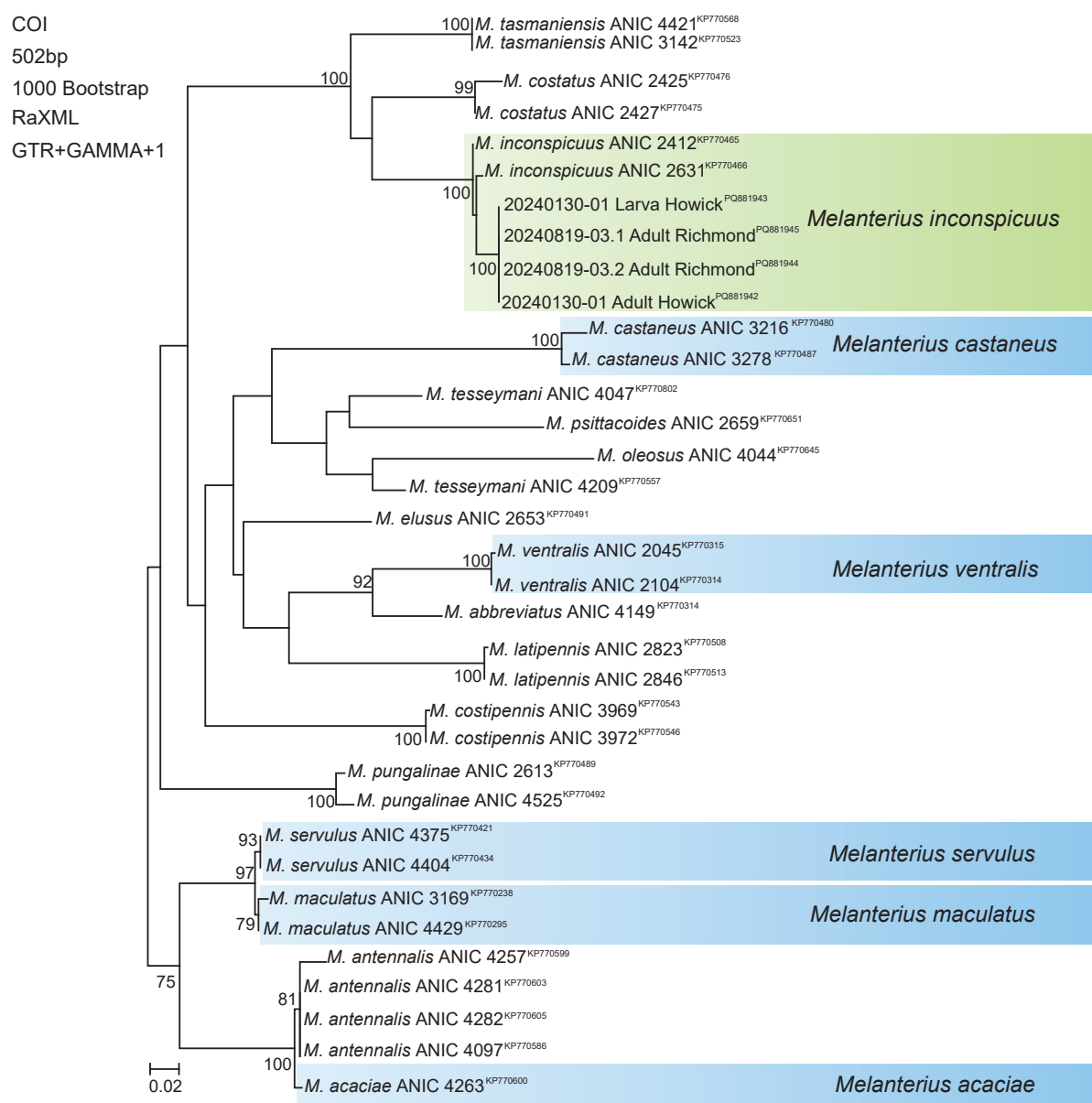


Figure 2: Maximum-likelihood phylogram of mitochondrial COI sequences showing phylogenetic placement of the six *Melanterius* species present in South Africa (support values above 75 presented at nodes; sequences identified by their ANIC accession and GenBank numbers (superscript); blue blocks denote the five species introduced into South Africa as biological control agents; green block denotes the newly identified *Melanterius inconspicuus*)

trees. Swelling of the branches had also persisted, but only exit holes were visible (Figures 3e, 3f) and no larvae were found in the tissues of the shoots. However, adult weevils were discovered sheltering in bark crevices and at sites of hail and mechanical damage to the trees (Figure 3d).

In the follow-up investigation in August 2024, no larvae were found on the now one-year-old trees at Site 1 compared to earlier in the year. However, the feeding of the larvae in the stems and shoots had resulted in swollen and callused tissues around their tunnels (Figure 3e). Splitting and cankering of the shoots was prevalent, likely a result of both the swelling of the tissues induced by the larval feeding and the damage induced during emergence of the larvae

before pupation (Figure 3f). At both the original sites visited and the site in the Richmond area, adult weevils were again encountered sheltering in the cervices and hail-damaged areas of the stems and branches of the trees.

Discussion

This study presents the first report of the non-native weevil *M. inconspicuus* in South Africa, affecting *A. mearnsii*. The identification of the species was based on both morphological characterisation and molecular phylogenetic analyses. This species has so far been detected on five *Acacia* species in its native range in Australia, whereby only one of these

detections (on *A. dealbata*) was deemed to signify a putative host association and the others (on *A. decurrens*, *A. fulva*, *A. fimbriata* and *A. irrorata*) considered merely coincidental occurrences (Pinzón-Navarro et al. 2017 Table 2). The discovery of larvae of *M. inconspicuus* inside the shoots of *A. mearnsii* represents the first definite host association reported for this species and the first record of a *Melanterius* species found feeding in the vegetative parts of a host plant as opposed to its seeds. In addition, this represents the first record of any *Melanterius* species established outside of its native range in Australia, apart from the five species intentionally introduced in South Africa as biological control agents and *M. elusus*, which was initially recorded from the USA but is not known to be established there (Anderson 2008; Pinzón-Navarro et al. 2017).

Melanterius inconspicuus is a rather uncommon species in its native Australia, with only few records of its occurrence available. Only seven specimens are housed at ANIC, four collected before 2000 and three collected in 2012. A few additional records by M van den Berg and S Naser also exist (Pinzón-Navarro et al. 2017), and some more specimens are likely present in other collections in the country, albeit probably unidentified.

The discovery of larvae of *M. inconspicuus* in the vegetative tissues of *A. mearnsii* in South Africa is particularly interesting given what is generally known about the life cycles of these weevils. Nothing specific has been reported about the life cycle and larval development of *M. inconspicuus* in Australia, apart from a short series of adults having been found in *Uromycladium* rust galls on *Acacia* (Pinzón-Navarro et al. 2017). This habit has also been recorded for the related *M. costipennis*, although it is not known whether the larvae of either species may develop in these galls (Pinzón-Navarro et al. 2017). Old galls of *Uromycladium morrisii* on *A. saligna* are used as an overwintering refuge by adult weevils of *M. castaneus* in both Australia and South Africa, although its larvae only develop in the seed pods (F Impson, personal observations). Whilst additional research is required to further investigate the occurrence of these weevils in vegetative tissues, these findings suggest that the larvae of at least some *Melanterius* species may be shoot-borers rather than just strictly seed-feeders. Although the development and emergence of the larvae caused damage to the shoots and stems, the evident recovery of the trees from January to August suggests that the weevils do not currently have a major effect on the general health of the young trees. However, continued monitoring and observations will be necessary to determine what effect, if any, the weevils may have on these trees in the long term.

In addition to finding the larvae of *M. inconspicuus* in the stems of *A. mearnsii*, many adults of the species were discovered on older branches and stems, aggregating in crevices or in lesions caused by hail or mechanical damage. Their finding during both the January and August field visits is consistent with the behaviour of sexually immature adults of other *Melanterius* species, which typically appear from January to August in Australia and South Africa (Impson and Hoffmann 2019). Further investigation into these weevils will be necessary to fully unravel their life history and behaviour as well as any effects they might have on wattle production in South Africa.

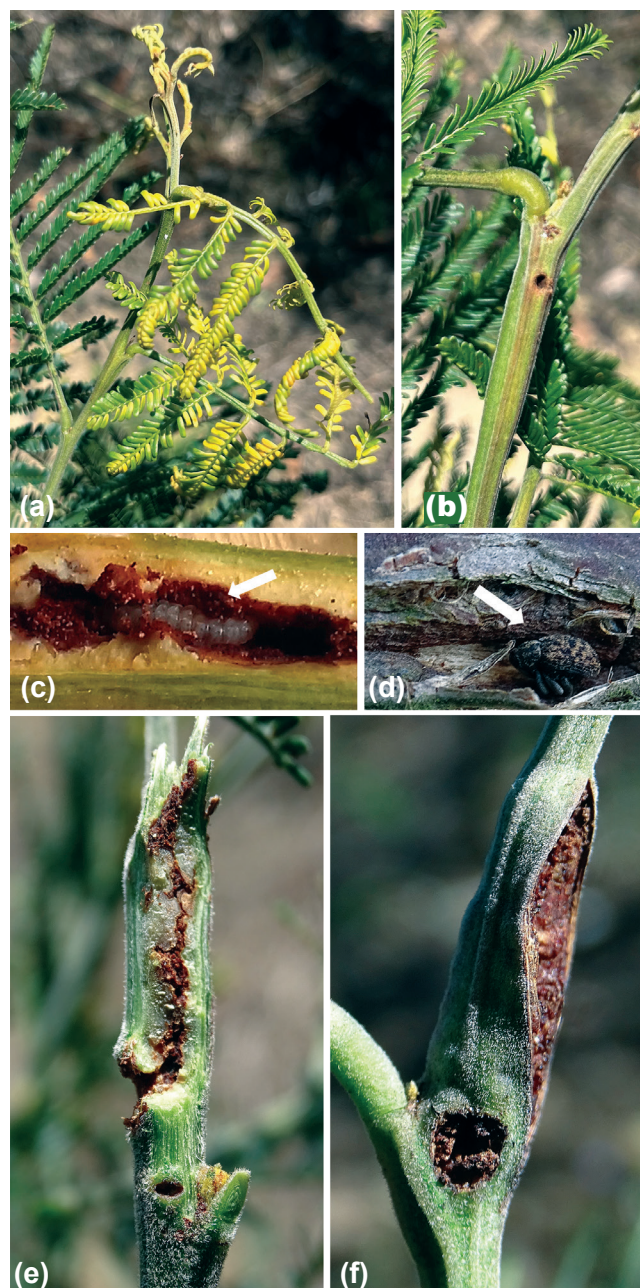


Figure 3: Symptoms of the activities of *Melanterius inconspicuus* on *Acacia mearnsii* trees in the KwaZulu-Natal Midlands: (a) yellow flagging and curling of shoots and growing tips; (b) emergence holes with slight swelling of the shoot; (c) larva (arrow) tunnelling in shoot; (d) adult weevil (arrow) found in a stem wound caused by hail; (e) callused and swollen shoot around a developmental chamber of a larva; (f) emergence hole and splitting of the stem caused by larval activity

Conclusions

Unlike plantations of *Pinus* and *Eucalyptus*, plantations of *Acacia* in South Africa have been relatively unperturbed by the accidental introduction of insects from their native range of Australia (Crous et al. 2017; Hurley et al. 2023). In fact, this is the first report of an *Acacia*-infesting insect species

from Australia on *A. mearnsii* in South Africa, aside from the purposely introduced biological control agents (Impson et al. 2023) and the polyphagous scale insect *Icerya purchasi* (Hemiptera: Margarodidae), which occasionally also attacks *A. mearnsii* (Kumschick and Jansen 2023). Although our initial observations suggest that *M. inconspicuus* is not currently a major threat to *A. mearnsii* in plantations, its detection raises concern about the pathway of its introduction. However, monitoring is ongoing to establish the true threat that this weevil poses to *Acacia* forestry in South Africa.

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