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Rapid Communication

First record of an African grass-feeding wasp (*Tetramesa*; Eurytomidae) on the invasive grass *Eragrostis curvula* (African lovegrass; Poaceae) in Australia

Guy F. Sutton[1,*], Clarke J.M. van Steenderen[1], Liam D. Yell[1], Kim Canavan[1], Andrew McConnachie[2] and Iain D. Paterson[1]

[1]*Centre for Biological Control, Department of Zoology and Entomology, Rhodes University, Makhanda, 6140, South Africa*
[2]*Weed Research Unit, Invasive Species Biosecurity, New South Wales Department of Primary Industries, Orange, 2800, Australia*
**Corresponding author*
E-mail: *g.sutton@ru.ac.za*

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Abstract

An undescribed phytophagous wasp belonging to the *Tetramesa* genus (Hymenoptera: Eurytomidae), that is native to South Africa, is currently being investigated as a potential weed biological control agent for the invasive grass *Eragrostis curvula* (Poaceae) in Australia. Host-specificity testing is underway in South Africa, but the wasp has not been exported into quarantine in Australia and further research is required before it could be considered for release. Here, we used DNA barcoding to demonstrate that *Tetramesa* specimens collected on invasive *E. curvula* populations in Australia represent the same wasp species currently being investigated in South Africa. We discuss our findings in the context of developing a biological control programme against *E. curvula* in Australia and the potential risk posed to native Australian grasses.

Key words: biocontrol, grass invasion, invasive insects, accidental introduction, natural enemies

Introduction

The stem-galling wasp genus *Tetramesa* Walker (Hymenoptera: Eurytomidae) is a cosmopolitan genus of 204 described species, and numerous presently undescribed species (Sutton et al. 2021). The larvae of *Tetramesa* are phytophagous, endophagous borers of cereals and other grasses (Poaceae) (Claridge 1961). Most *Tetramesa* have a narrow host range, with most species being recorded from a single genus of host plants (Claridge 1961; Goolsby and Moran 2009; Sutton et al. 2021). *Tetramesa* spp. can also have significant impacts on plant productivity, with several species considered important cereal crop pests (Spears and Barr 1985). Due to their high degree of host specificity and potential to reduce plant fitness, *Tetramesa* have been identified as high priority biological control agents for grasses that have become invasive in non-native geographic regions across the globe (Goolsby and Moran 2009; Sutton et al. 2019; Olckers et al. 2021).

Since 2017, the Centre for Biological Control (CBC) at Rhodes University in South Africa has performed extensive field surveys and laboratory trials

in South Africa investigating numerous *Tetramesa* as biological control agents for African grasses that have become invasive in Australia or the USA, namely: *Sporobolus pyramidalis* Beauv., *Sporobolus natalensis* (Steud.) Durand & Schinz, *Eragrostis curvula* (Schrad.) Nees, *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs and *Andropogon gayanus* Kunth (Sutton et al. 2019, 2021; Olckers et al. 2021). Little is known about the *Tetramesa* occurring in Africa, with only four described species from the continent: *T. aristidae* Risbec from Senegal, *T. decaryi* Risbec from Madagascar, *T. tananarivense* Risbec from Madagascar, and *T. macalusoi* De Stefani from Somalia (van Noort 2022). No formally described *Tetramesa* are known from South Africa other than an adventive population of *Tetramesa romana* Walker found on the invasive reed *Arundo donax* L. (Poaceae) (Canavan et al. 2014). As such, all the *Tetramesa* species being investigated for biological control in South Africa remain undescribed. However, DNA barcoding has allowed us to distinguish among different putative novel species of *Tetramesa* in South Africa and assess their host range and potential as biological control agents. These DNA phylogenies will also assist with the formal description of these species by relevant taxonomic experts, which is currently underway.

Eragrostis curvula is considered an invasive weed in Australia and a biological control programme has been initiated. Surveys for potential agents have been conducted in South Africa resulting in the discovery of an undescribed *Tetramesa* species associated with the target plant (Olckers et al. 2021). The same *Tetramesa* species, confirmed with molecular barcoding, has been reared from at least six native non-target *Eragrostis* species under field conditions in South Africa, namely: *E. capensis* (Thunb.) Trin., *E. plana* Nees, *E. gummiflua* Nees, *E. biflora* Hack., *E. cylindriflora* Hochst. (=*E. rigidior* Pilg.) and *E. trichophora* Coss. & Durieu (Sutton et al. *unpublished*). Laboratory no-choice testing has also demonstrated that this *Tetramesa* species is able to complete development and produce F_1 progeny on at least two native African *Eragrostis* species, namely: *Eragrostis planiculmis* Nees and *Eragrostis plana* Nees (Sutton et al. *unpublished*). It is therefore questionable whether this particular *Tetramesa* species is suitably host specific for release in Australia, and additional host-specificity testing against several native Australian *Eragrostis* species is currently being performed under quarantine conditions in South Africa to assess this.

Ad hoc surveys by two of the authors (GFS, AM) across New South Wales, Victoria and South Australia, constituting a large portion of the invaded distribution of *E. curvula* in Australia (Figure 1) were conducted in September 2022, and resulted in the discovery of a *Tetramesa* sp. attacking invasive populations of *E. curvula* (Figure 2). The wasp was recorded at six out of eight sites visited, being absent from the two field sites in South Australia (Figure 1). Where present, the wasp was found in high numbers

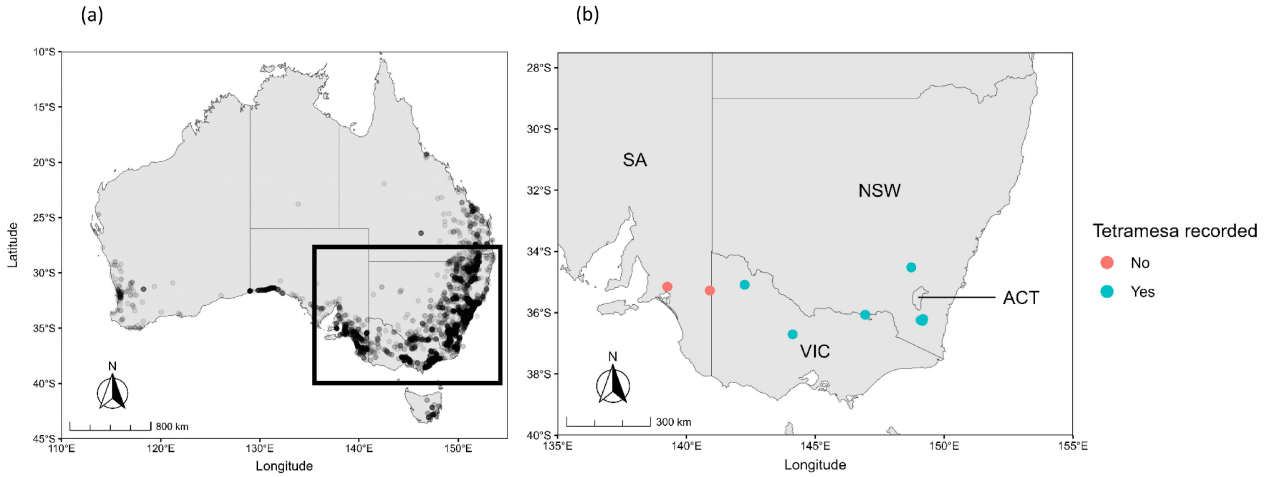

Figure 1. Maps indicating (a) the distribution of invasive populations of *Eragrostis curvula* in Australia (black bounding box indicates the geographic extent of the map shown in panel (b), and (b) the sites sampled for *Tetramesa* in October 2022, whereby blue filled dots indicate sites where *Tetramesa* was recorded, and pink filled dots indicates sites where *Tetramesa* was not recorded. *Eragrostis curvula* records in Australia were downloaded from the Global Biodiversity Information Facility using the R package '*rgbif*' (Chamberlain et al. 2022; GBIF Occurrence Download 2022).

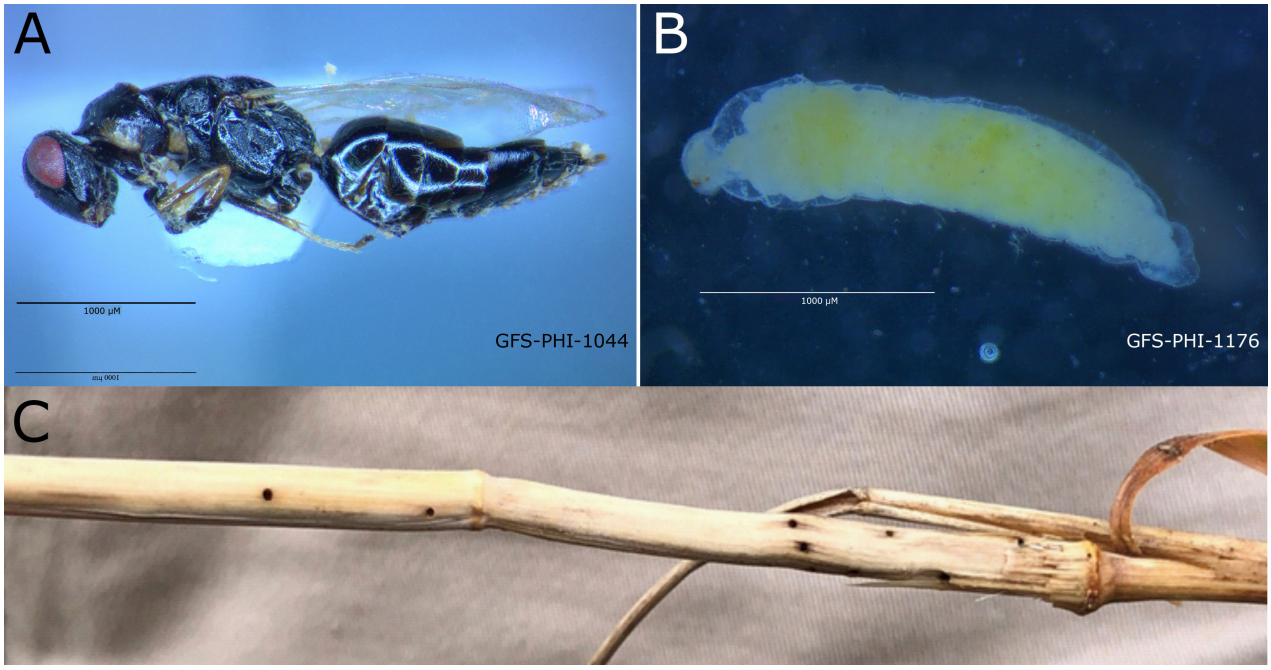

Figure 2. *Tetramesa* A) Adult female (note the characteristic "golden" pronotal spot), B) larva collected on *Eragrostis curvula* in Australia, and C) characteristic emergence holes located above the 1st and/or 2nd tiller node Scale bars represent 1 mm.

across all different life-stages (larvae, pupae and adults). Field observations indicated that the wasps were causing damage to the host plants, with infested grass tillers showing severe stem deformation, and in many cases, wasps appear to have stopped stem elongation and inflorescence formation entirely, which prevents seed set. The presence of *Tetramesa* can be rapidly assessed by characteristic emergence holes located above the 1st and 2nd tiller nodes (Figure 2). To date, only one *Tetramesa* species is known from Australia, namely *Tetramesa australiensis* Girault (=*Isosoma australiensis*) (Girault 1913). It was described from a single male specimen collected

using a sweep net in Kuranda in northern Queensland, and as such, no host-plant records are available (Girault 1913). It is however likely that other *Tetramesa* species may be present and indigenous in Australia that are undescribed. Given the high degree of host specificity exhibited by most *Tetramesa* species, and the non-native status of *E. curvula* in Australia, it is plausible that these wasps collected in Australia may be the same species of *Tetramesa* that is recorded on the plant in South Africa.

The aim of this study was to document the occurrence of the undescribed *Tetramesa* species on *E. curvula* in Australia, and evaluate whether the wasps may represent a previously unknown introduction of the South African *Tetramesa* sp. currently being assessed as a biological control agent or if it is a novel association between the invasive grass and a native Australian *Tetramesa*. The morphology of both adults and larvae of *Tetramesa* is extremely cryptic, with little morphological variation present to allow for distinction of different species. Moreover, the lack of a taxonomic backbone for *Tetramesa* in both Africa and Australia largely negates the use of traditional morphological taxonomy for this group. As such, we compared cytochrome oxidase I (COI) gene sequences for South African *Tetramesa* associated with native African *Eragrostis* species, with sequences generated for representative *Tetramesa* samples collected on *E. curvula* in Australia. We discuss our findings in the context of developing a biological control programme against *E. curvula* in Australia.

Methods

Grasses showing signs of possible *Tetramesa* infestation were collected from field sites across southern Africa and Australia. Wasps were extracted from these grasses either by dissection using a fine scalpel or were placed in standard insect emergence chambers. At least one representative specimen was included in the current study from all 16 grass species from which *Tetramesa* specimens have been collected in South Africa, to date (see Supplementary material Table S1). Five representative *Tetramesa* specimens were also included that were collected from *E. curvula* plants sampled in Australia (Table S1). The *Tetramesa* COI dataset (n = 115 sequences) included five wasp sequences collected from *E. curvula* in Australia, 37 sequences collected from four populations of *E. curvula* collected across South Africa, 31 sequences collected from seven congeneric *Eragrostis* grasses across South Africa, and 38 sequences from non-*Eragrostis* grasses sampled across southern Africa.

Genomic DNA extractions were performed using a PureLink™ Genomic DNA Mini Kit (ThermoFisher Scientific, catalogue number K182002) according to the manufacturer's protocols. Mitochondrial cytochrome c oxidase I (COI) sequences were obtained using the forward FWPTF1 (5' CCT GGTTCTTTRATTGGTAATGATC 3') and reverse LepR1 (5' TAAACTTC TGGATGTCCAAAAA 3') primers (Li et al. 2010). Successfully amplified

PCR products were sent to Macrogen Inc. in the Netherlands for post-PCR purification and sequencing in the reverse direction. The reverse direction was chosen to avoid the poly-T region at the beginning of the forward sequence that interrupts downstream sequencing. Sequences were subsequently reverse complemented and aligned online using the default parameters in MAFFT v7 (https://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019).

Bayesian phylogenies were created using MrBayes v3.2.6 (Huelsenbeck and Ronquist 2001) on the CIPRES Science Gateway v3.3 online server (http://www.phylo.org/) (Miller et al. 2010), using random starting trees, four chains (three hot and one cold), two runs, and seven million generations. Posterior probability values between 0.9–1.0 were considered strongly supported. Three *Sycophila* sequences served as outgroups (GenBank codes AY317230–AY317232) (Chen et al. 2004).

Results

The Bayesian phylogeny indicated that the *Tetramesa* collected from all the *Eragrostis* grasses, in both South Africa and Australia, form a well-supported clade that is sister to undescribed *Tetramesa* specimens collected from other African grass genera such as *Sporobolus*, *Eustachys*, *Hyparrhenia* and *Andropogon*, as well as the only two described *Tetramesa* species with COI sequences available online, namely: *Tetramesa romana* and *Tetramesa bambusae* Philips (Figure 3). All the Australian *Tetramesa* specimens collected from *E. curvula* formed a well-supported clade (Clade E) with wasps collected from *E. curvula* in the Free State Province of South Africa (Figure 3).

Discussion

The CO1 phylogeny clearly indicated that the *Tetramesa* on *E. curvula* in Australia is of African origin and was most likely introduced along with its host plant. This introduction could have occurred as early as the 1920's (*E. curvula* was recorded as naturalized in New South Wales, Victoria and South Australia by 1920; Australasian Virtual Herbarium 2023). It is possible that undiscovered *Tetramesa* are associated with Australian *Eragrostis* spp., but these would most likely be genetically distinct from African species and would certainly have fallen outside of any *E. curvula* clade of wasps from South Africa. This would not be the first time a *Tetramesa* species has been introduced with its host plant into a non-native geographic region. The Arundo wasp *T. romana* has been recorded on its host plant *Arundo donax* L. in several countries around the world where *A. donax* has been introduced, such as the USA and South Africa (Goolsby and Moran 2009; Canavan et al. 2014). Although damaging genotypes of *T. romana* were introduced as a biological control agent to the USA, an adventive genotype of the same wasp species was already present prior to these introductions (Dudley et al. 2006). *Tetramesa* species typically overwinter inside the grass tiller, and can

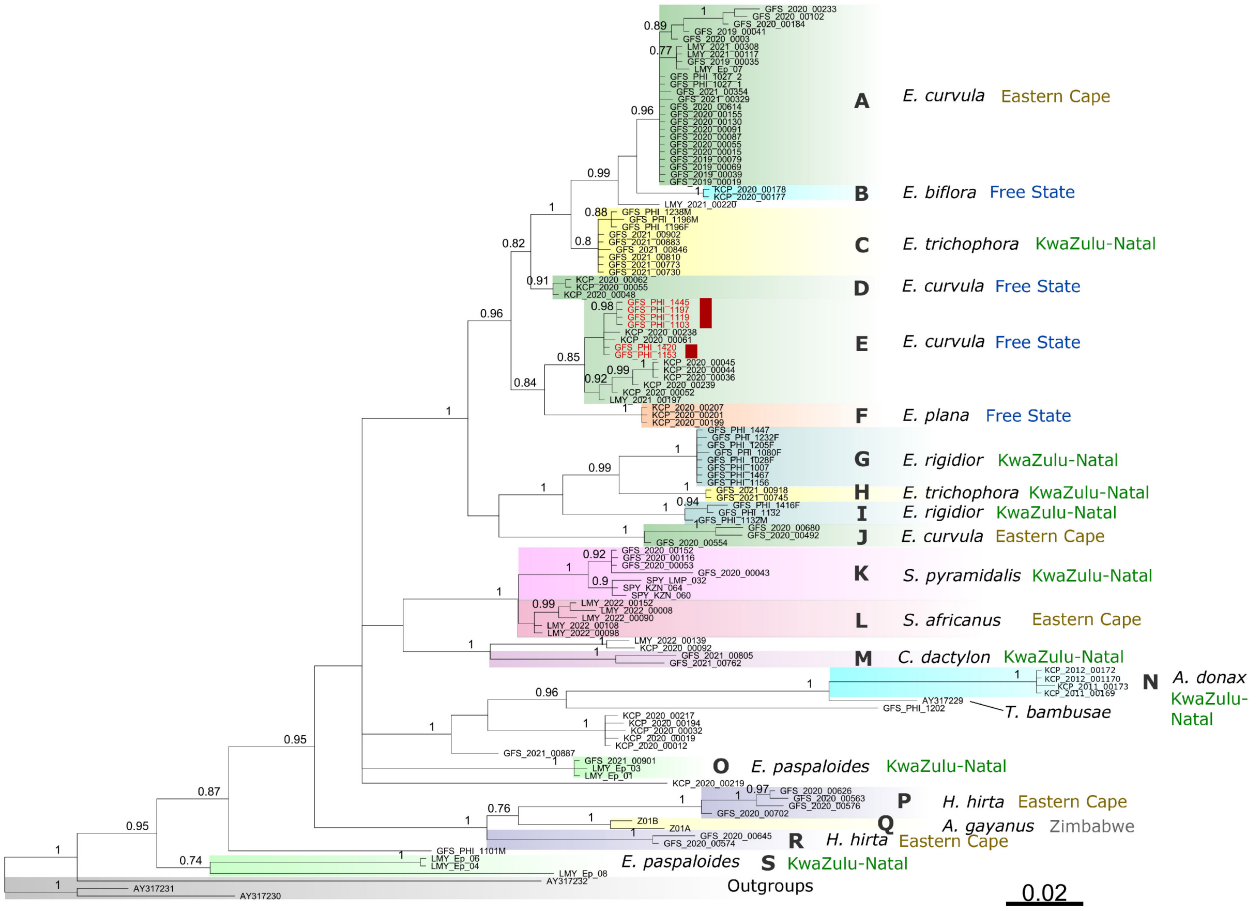

Figure 3. A Bayesian COI phylogeny showing the major *Tetramesa* clades present in South Africa, the host plants of each clade and the South African province where the samples from the clade were collected. The *Tetramesa* collected on *Eragrostis curvula* in Australia are shown in red text, marked with red rectangles in clade E. The scale bar represents the number of nucleotide substitutions per site. Posterior probability values are shown above the branches.

emerge from tillers that have been excised from the grass tussocks for at least four months (Sutton *pers. obs.*), which would allow them to survive being transported to Australia from South Africa as live plants, packing material, fodder or hay bales, as a possible modes of introduction.

The *Tetramesa* sp. found in Australia has been reared from at least six native *Eragrostis* species under field conditions in South Africa and produce F_1 progeny on at least two native African *Eragrostis* (*E. plana* and *E. planiculmis*) under controlled greenhouse no-choice conditions (Sutton et al. *unpublished*). The host specificity of this *Tetramesa* sp. therefore appears to be confined to a number of closely-related *Eragrostis* species, which is in contrast to the majority of *Tetramesa* species that are typically monophagous (Goolsby and Moran 2009; Sutton et al. 2019, 2021). There are more than 70 native *Eragrostis* species in Australia, several of which, such as *E. pilosa* (L.) P. Beauv., *E. leptocarpa* Benth., and *E. leptostachya* (R. Br.) Steud, amongst others, are as closely (or more closely) phylogenetically related to *E. curvula* than either *E. plana* or *E. planiculmis* (Barrett et al. 2020). Given that insect herbivore host range is typically strongly linked to phylogenetic distance between the host plants (Wapshere 1974), it is possible that several Australian native *Eragrostis* species may be at risk of attack by this *Tetramesa* sp. Field surveys of the native *Eragrostis* species that are closely related to *E. curvula*

should be conducted across Australia to determine how widespread the wasp is and if it is impacting native *Eragrostis* populations. In the meantime, the *Tetramesa* sp. recorded on *E. curvula* in Australia has been reported to the Australian Chief Plant Protection Officer and the Consultative Committee on Emergency Plant Pests.

The *Tetramesa* sp. is also likely to be providing benefits in Australia by damaging the invasive alien populations of *E. curvula.* These impacts need to be quantified but, given that the most promising candidate agent has been discovered in the invaded distribution, the need to release this agent may have been negated. The relative impacts of the South African *Tetramesa* populations that were being considered for biological control could be compared to the impacts of the Australian *Tetramesa* populations to determine whether more damaging genotypes should be considered for release, as was the case for the biological control of *A. donax* using *T. romana* in the USA (Goolsby and Moran 2009). It would, however, be important to complete host-specificity testing of the South African *Tetramesa* populations before they are considered for release because the preliminary results do suggest that the host range of the insect may be too broad for consideration for release in Australia.

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Authors' contribution

GFS: Research Conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, writing – original draft, writing – review and editing. CVS: Investigation and data collection, data analysis and interpretation, writing – original draft, writing – review and editing. LDY: Investigation and data collection, sample design and methodology, writing – review and editing. KC: Research Conceptualization, investigation and data collection, writing – review and editing. AM: Research Conceptualization, investigation and data collection, funding provision, writing – review and editing. IP: Research Conceptualization, data analysis and interpretation, funding provision, ethics approval, writing – review and editing.

Ethics and permits

The work performed in the current study is performed under the ethics approval no. 2022-3842-6516 (Rhodes University, South Africa).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Specimen information sheet for representative DNA samples included in the DNA barcoding study

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2023/Supplements/BIR_2023_Sutton_etal_SupplementaryMaterial.pdf