



Phylogenetic analyses reveal multiple new stem-boring *Tetramesa* taxa (Hymenoptera: Eurytomidae): implications for the biological control of invasive African grasses

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Abstract Many native South African grass species have become invasive elsewhere in the world. The application of biological control to invasive grasses has been approached with trepidation in the past, primarily due to concerns of a perceived lack of host specific herbivores. This has changed in recent times, and grasses are now considered suitable candidates. The *Tetramesa* Walker genus (Hymenoptera: Eurytomidae) has been found to contain species that are largely host specific to a particular grass species, or complex of closely related congeners. Very little taxonomic work exists for *Tetramesa* in the southern

hemisphere, and the lack of morphological variability between many *Tetramesa* species has made identification difficult. This limits the ability to assess the genus for potential biological control agents. Species delimitation analyses indicated 16 putative novel southern African *Tetramesa* taxa. Ten of these were putative *Tetramesa* associated with *Eragrostis curvula* (Schrud.) Nees and *Sporobolus pyramidalis* Beauv. and *S. natalensis* Steud., which are alien invasive weeds in Australia. Of these ten *Tetramesa* taxa, eight were only found on a single host plant, while two taxa were associated with multiple species in a single grass genus. The *Tetramesa* spp. on *S. pyramidalis* and *S. africanus* were deemed suitably host-specific to be used as biological control agents. Field host range data for the *Tetramesa* species on *E. curvula* revealed that the wasp may not be suitably host specific for use as a biological control agent. However, further host specificity testing on non-target native Australian species is required.

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Introduction

South Africa has been a major donor of invasive grasses to other parts of the world due to the perception that African C₄ grasses were more palatable to

livestock and resistant to grazing than native grasses (Visser et al. 2016). Many African grasses have subsequently become invasive and highly damaging due to the combination of favourable traits, multiple introduction events (D'Antonio and Vitousek 1992; Visser et al. 2016), and high propagule pressure (Firn 2009). Historically, very few grasses have been targeted using biological control, mainly due to concerns of potential non-target attacks on economically valuable crops, tolerance to herbivory, and the belief that there were few or no specialist herbivores on grasses (Sutton et al. 2021a). These perceptions are, however, changing with the discovery of host-specific taxa (Goolsby et al. 2020), particularly eurytomid wasps in the *Tetramesa* Walker (Hymenoptera: Eurytomidae) genus (Witt and McConnachie 2004; Sutton et al. 2021a,b).

At the time of the review of grass biological control by Sutton et al. (2021a), 23 invasive grass species were currently, or had already been, investigated as targets for biological control using herbivorous arthropods and fungal pathogens. The global weed biological control catalogue listed 14 releases of seven biological control agent species onto five invasive grass species between 1997 and 2019 (Winston et al. 2021; Supplementary Table S1). To date, *T. romana* Walker is the only *Tetramesa* species that has been used as a biological control agent for an alien invasive grass, namely *Arundo donax* L. (giant reed or carrizo cane) in the USA (Goolsby and Moran 2009; Moran and Goolsby 2009). *Tetramesa romana* is considered successful in the USA, particularly in the Rio Grande, Texas, where *A. donax* biomass was estimated to be reduced by 32% in 2016, equating to 4.4 million \$US a year in water savings (Goolsby et al. 2016; Moran et al. 2017). Following the success of this programme, there has been an increasing interest in the use of *Tetramesa* as potential biological control agents for other alien invasive grasses (Lotfalizadeh et al. 2020; Sutton et al. 2021b).

The *Tetramesa* genus currently comprises over 200 described species (Al-Barrak 2006; Lotfalizadeh et al. 2020; Natural History Museum 2021). They are highly host-specific, and typically specialise on only one species or genus, or on closely related genera, in the Poaceae (Phillips 1936; Claridge 1961). The larvae are endophagous feeders, and are either stem borers or gallers (Claridge 1961). Almost all of the sampling effort in collecting and describing *Tetramesa*

species has taken place in the Northern Hemisphere (Al-Barrak 2006) (Supplementary Fig. S1). Only four African species have been described, namely *T. aristidae* Risbec from Senegal, *T. decaryi* Risbec and *T. tananarivense* Risbec from Madagascar, and *T. macalusoii* De Stefani from Somalia (van Noort 2020). No *Tetramesa* species have been described yet from South Africa (van Noort 2020), despite the existence of novel taxa in the region (Witt and McConnachie 2004; Sutton et al. 2021b).

In addition to *T. romana*, a number of other *Tetramesa* species are currently being considered as potential grass biological control agents. Witt and McConnachie (2004) reported the presence of an unidentified *Tetramesa* sp. on *Sporobolus africanus* (Poir.) Robyns and Tournay, *S. natalensis* (Steud.) T. Durand and Schinz, and *S. pyramidalis* P. Beauv. in southern Africa. A second unidentified *Tetramesa* species was found on *S. natalensis* and *S. pyramidalis* by Sutton et al. (2021b) during native range surveys, where the wasp was found on only these two target weeds and not on any native congeners or other close relatives. Sutton et al. (2021b) found that both *Tetramesa* species significantly decreased tiller survival and had a deleterious impact on tiller reproduction. There are several other grasses that are indigenous to South Africa that are potential targets for biological control that may have other *Tetramesa* species associated with them, particularly *Eragrostis curvula* (Schrad.) Nees and *Andropogon gayanus* Kunth (Olckers et al. 2021; Sutton et al. 2021b).

The Centre for Biological Control (CBC) at Rhodes University, South Africa, has been conducting field surveys for natural enemies on native grasses since 2017 across more than 200 sites and 70 grass species. The morphological uniformity of the adult and larval stages of the *Tetramesa* makes it extremely challenging to identify different species (Dawah 1987; Ghajarieh et al. 2006). Even genus-level identifications delineating between the *Tetramesa* and *Eurytoma* have proven difficult (Henneicke et al. 1992), and reliable synapomorphies at even the family-level are still lacking (Lotfalizadeh et al. 2007; Gates 2008). The lack of a taxonomic backbone for *Tetramesa* in South Africa and suitable morphological tools to distinguish between difference species has impeded our ability to assess the host specificity of field collected specimens and to establish pure laboratory cultures. The aims of the present work were

therefore to use genetic techniques to distinguish between the *Tetramesa* species, and from this make inferences about their predicted host ranges. Delineating species in this morphologically cryptic genus will advance efforts to identify potential biological control agents for the African grasses that have become invasive elsewhere.

Materials and methods

Sample collection

Wasp samples for DNA analysis were collected from six South African provinces and 19 grass host plants (Supplementary Fig. S2, Fig. S3, Table S2, Table S3). Surveys were performed through either stem dissections of collected grasses or emergence chambers in the laboratory to ensure that the host-plant record was a true interaction and not due to chance. Voucher specimens were identified to the lowest taxonomic level by experts at the South African National Insect Collection in Pretoria (ARC-PPRI).

Host grasses were selected for sampling based on their invasive status and prioritisation in biological control programmes in Australia and the USA. The main targets were *Andropogon gayanus*, *Eragrostis curvula*, *Megathyrsus maximus* (= *Panicum maximum*) (Jacq.) B.K. Simon and S.W.L. Jacobs, *Sporobolus natalensis*, and *S. pyramidalis*, all of which are serious alien invasive pests in Australia and the USA. *Hyparrhenia hirta* (L.) Stapf was included because this grass is also a problem species in Australia and might be considered for a future biological control programme (Sutton et al. 2019).

Sample preservation and DNA extraction

All insect specimens were stored in 95% ethanol at -20°C . Reference specimens were photographed under a ZEISS Stemi 508 stereo microscope using ZEN imaging software [ZEN Digital Imaging for Light Microscopy (RRID:SCR 013672)], and CombineZP software for z-stacking. A full database of photographs is housed on the associated GitHub project repository (https://github.com/clarkevans teenderen/PhD_files/tree/main/Microscope%20Images). Three legs from each specimen were removed under a dissecting microscope so that the remaining

whole specimens could be used for direct comparisons after sequencing. Genomic DNA extractions were performed using a PureLink™ Genomic DNA Mini Kit (ThermoFisher Scientific, catalogue number K182002) according to the manufacturer's protocols. Supplementary File S1 provides details of the polymerase chain reaction (PCR) protocols, nucleotide sequencing and alignments, phylogenetic analyses, and Generalised Mixed Yule Coalescent (GMYC) species delimitation analysis.

Results

Both the COI (log-likelihood of the ML consensus tree = -10200.798 , BI run 1 = -10641.18 , BI run 2 = -10661.60) and 28S (log-likelihood of the ML consensus tree = -3840.301 , BI run 1 = -4097.81 , BI run 2 = -4115.02) phylogenies (see clade name abbreviations in Supplementary Table S4) showed a distinct separation between pronotal spot (PNS) and non-pronotal spot (NPNS) eurytomids, but the NPNS groups were unresolved (Figs. 1, 2, Supplementary Fig. S5, Fig. S6, Fig. S7).

28S data

Overall inter- and intra-specific p-distances (excluding the outgroup) ranged from 3 to 9.7% and 0 to 2.7%, respectively. Sequence divergences within the southern African PNS groups ranged from 0.6 to 1.9% (Supplementary Table S5 and Fig. S8). The only three well-defined clades within the PNS group were the wasps collected from *Eragrostis rigidior* (PNS. ERIG.1; clade 1, posterior probability (pp)=0.71), *Sporobolus* spp. (PNS.SPOR.1; clade 2, pp=0.84, bootstrap support (bt)=91), and *Arundo donax* (PNS. ADON.1, clade 3, pp=1, bt=100) (Fig. 1, Supplementary Fig. S5). The nuclear marker showed that both the Eurytominae subfamily and the proposed *Tetramesa* formed well-supported monophyletic groups (Fig. 1, Supplementary Fig. S5, pp=1). These results suggest that a sequence divergence of ~ 3 to 3.5% and above, likely does not belong to the *Tetramesa* genus (Supplementary Table S5), and that major groups within the PNS clade can be delineated at a threshold of below 0.4%. The only NPNS wasps that fell within the proposed *Tetramesa* group were those collected from *H. hirta* (NPNS.HHIR.1)

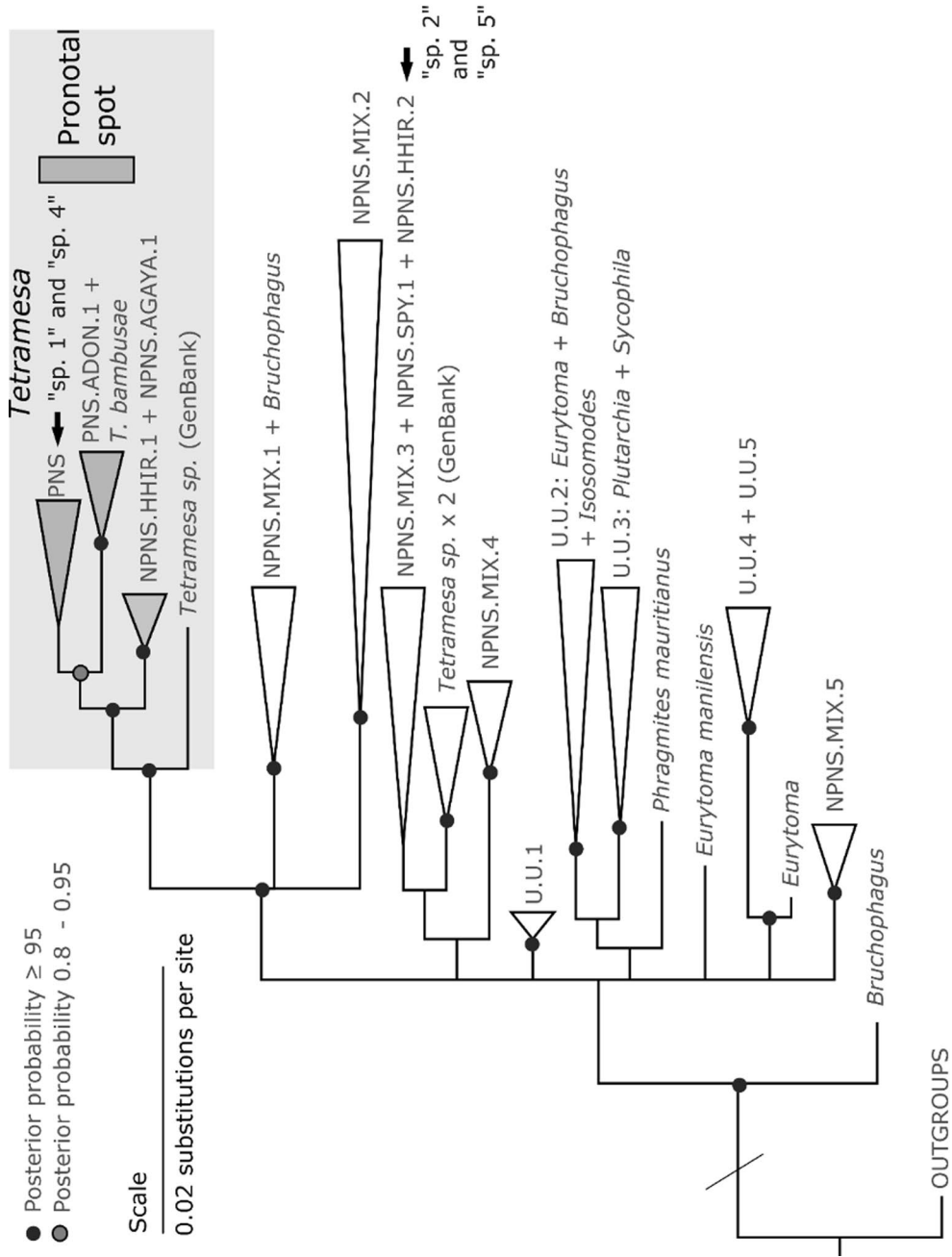


Fig. 1 Condensed Bayesian inference (BI) phylogeny for the 28S nuclear gene region. Posterior probability support values are shown as filled circles on the branches (see the legend). Supplementary Fig. S5 shows the full phylogeny with all the sample names displayed, and GMYC species delimitation results included in the figure. See the list of phylogenetic clade abbreviations in Supplementary Table S2

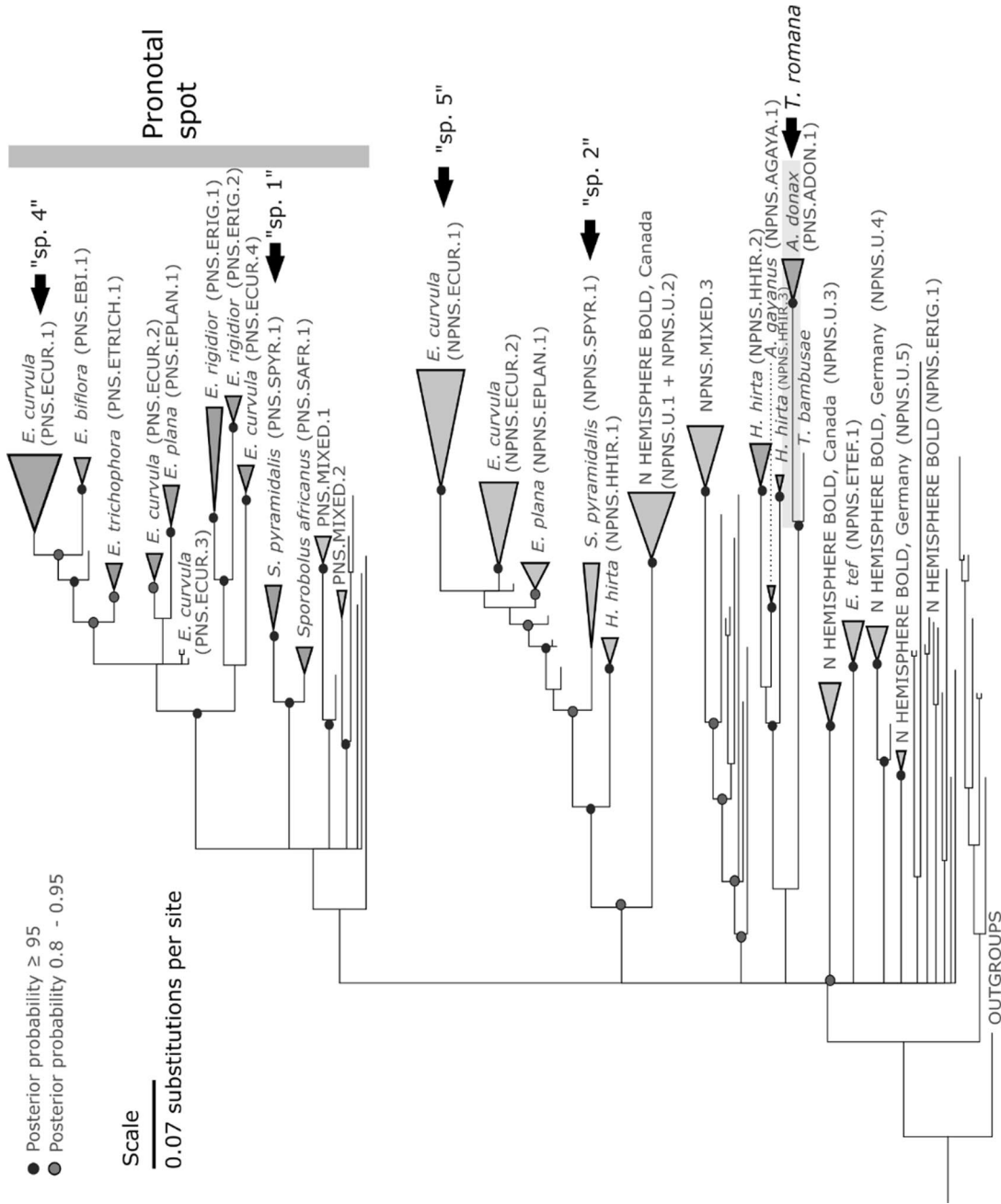


Fig. 2 Condensed Bayesian inference (BI) phylogeny for the COI mitochondrial gene region. Posterior probability support values are shown as filled circles on the branches (see the legend). Supplementary Fig. S6 shows the full phylogeny with all the sample names displayed, and GMYC species delimitation results included in the figure. See the list of phylogenetic clade abbreviations in Supplementary Table S2

and *A. gayanus* (NPNS.AGAYA.1) (Fig. 1, Supplementary Fig. S5). The GMYC species delimitation method estimated 36 entities (95% CI=20–43) and 23 clusters (95% CI=15–28) (log-likelihood of the null model=957.25, ML of the GMYC model=963.6845, $p < 0.01$, singletons=13). Guided by the GMYC results, we estimated the presence of six host-specific taxa, namely PNS.ERIG.1, PNS.SPOR.1, PNS.ADON.1, NPNS.HHIR.1, NPNS.AGAYA.1, and NPNS.HHIR.2 (Fig. 1, Supplementary Fig. S5, Table S6).

COI data

Overall inter-group and intra-group p -distances (excluding the outgroup) ranged from 1.6 to 26.3% and 0 to 3.2% respectively. Chen et al. (2004) reported interspecific distances ranging from 0.2 to 25.8%. Most of the clades in the COI phylogeny were unresolved, rendering taxonomic inferences based on this marker very challenging (Fig. 2, Supplementary Fig. S6). Sequence divergences within the southern African PNS groups ranged from 2.4 to 13.1% (Supplementary Fig. S9). A sequence divergence of 2.8% was found between the PNS.ECUR.1 (PNS wasps on *E. curvula*) clade and its sister group PNS.ETRICH.1 (PNS wasps on *E. trichophora*) (Fig. 2, Supplementary Fig. S6), where the latter forms unique galls on *E. trichophora* that have not been observed on other grasses (Supplementary Fig. S10).

There were four separate clades associated with *E. curvula* within the PNS group (PNS.ECUR.1 PNS.ECUR.4), although only PNS.ECUR.1 ($pp=0.68$, $bt=95$) and PNS.ECUR.4 ($pp=1$, $bt=100$) were strongly supported (Fig. 2, Supplementary Fig. S6). The sequence divergence between PNS.ECUR.1 (Eastern Cape) and PNS.ECUR.2 (Free State) was 4.5% (Fig. 2, Supplementary Fig. S6, Fig. S9). The two *E. rigidior* clades, PNS.ERIG.1 and PNS.ERIG.2, were collected in the same geographic region, and had a sequence divergence of 7.1% (Supplementary Fig. S9).

The wasps collected on *Sporobolus pyramidalis* and *S. africanus* formed two distinct sister clades, with a sequence divergence of 3.9% (Fig. 2, Supplementary Fig. S6; PNS.SPYR.1; $pp=1$, $bt=89$ and PNS.SAFR.1; $pp=0.73$, $bt=99$). Additionally, the *S. pyramidalis* wasps showed some evidence of geographic substructuring between the

KwaZulu-Natal ($pp=0.9$, $bt=97$) and Eastern Cape ($pp=0.88$, $bt=95$) specimens. The GMYC results estimated 71 entities (95% CI=62–77) and 42 clusters (95% CI 36–43) (log-likelihood of the null model=1026.2, ML of the GMYC model=1082.666, $p < 0.001$, singletons=29). Based on the phylogenetic and GMYC analyses, we estimated the presence of thirteen host-specific taxa, namely PNS.ETRICH.1, PNS.EPLAN.1, PNS.ECUR.3, PNS.ERIG.2, PNS.ECUR.4, PNS.SPYR.1, PNS.SAFR.1, NPNS.ECUR.2, NPNS.HHIR.1, NPNS.HHIR.2, NPNS.AGAYA.1, NPNS.HHIR.3, and PNS.ADON.1 (Fig. 2, Supplementary Fig. S6, Table S6).

Discussion

In general, very little taxonomic work has focused on Afro-tropical insects, particularly micro-Hymenoptera such as the *Tetramesa* (van Noort et al. 2015; Berry and van Noort 2016; Hopkins et al. 2019). This investigation has provided new insights into the *Tetramesa* assemblages on native African grasses by investigating their diversity, phylogenetic relationships, and host specificity. The work presented here has identified multiple *Tetramesa* taxa that hold potential as biological control agents of invasive grasses, but some groups may be more suitably host-specific than others, and care must be taken to use an integrative taxonomic approach rather than relying solely on one line of evidence for identification.

Biological control outlook

The NPNS clades were found largely to be oligophagous feeders, and had a wider host range compared to the PNS groups. Biological control efforts should therefore prioritise the latter, and those that form part of the NPNS *H. hirta* and *Andropogon gayanus* clade, as well as PNS *T. romana*. This is based on the host-use patterns seen particularly in the COI phylogeny, where, with a few minor exceptions, each PNS clade was associated with a single host grass. The prospects for the biological control of the various target weeds are discussed below in the context of the phylogenies and inferences of host ranges. A promising species for *M. maximus* was found, but preliminary host rearing trials have indicated that this wasp can reproduce on

Setaria sphacelata (Schumach.) Stapf and C.E. Hubb (unpublished data, Guy Sutton).

Eragrostis

The PNS *E. curvula* wasps (morphospecies ‘*Tetramesa* sp. 4’) belonged to the *Tetramesa* genus, but are likely not good candidates for biological control due to their broader host range on conspecific *Eragrostis* species. These could include non-target Australian and North American natives, that are either more closely related to the target weed than to the congeners from which they were collected, or very closely related to the congeners.

The nine PNS clades associated with *Eragrostis* hosts identified in the present COI phylogeny (spanning *E. curvula*, *E. biflora*, *E. rigidior*, *E. trichophora*, and *E. plana*) were delineated into eight GMYC taxa, and may represent unique species. Further taxonomic confirmation is, however, required to validate this observation. The low sequence divergence between the wasps on *E. curvula* clades PNS.ECUR.1 – PNS.ECUR.3 and *E. trichophora* PNS. ETRICH.1 (ranging from p-distances of 2.4 – 3.9%) suggests that host-specificity testing of these two wasps is important, as each taxon may be able to utilise both host plants, which could preclude their use as biological control agents. No-choice host-specificity testing should be completed, as it seems unlikely, although not impossible, that the same species of wasp would have such different feeding modes on two different host plant species, namely galling *E. trichophora* and mining the stems of *E. curvula*. *Tetramesa* sp. 26 on *E. rigidior* (PNS.ERIG.1) may not be an appropriate biological control agent, as it was also recorded on *E. gummiflua* in the field and therefore has a relatively broad host range. The second *Tetramesa* sp. 26 clade (PNS.ERIG.2), however, may be useful as it was specific to only *E. rigidior*. Further sampling focusing on these species is required.

Further work should focus on a population genetics analysis of both the PNS *E. curvula* *Tetramesa* wasps and their hosts from a wider geographic range to test whether host plant form may be driving genetic divergence. *E. curvula* has been developed as a pasture grass and has had numerous varieties developed. Such genetic breeding has led to distinct forms of the grass that vary morphologically and chemically (e.g. ? phosphate and fibre concentrations) (Leigh 1961).

In the case of different *E. curvula* haplotypes in the native range, it will be vital to confirm matches to haplotypes in the invaded range to ascertain points of origin and select the most appropriate *Tetramesa* population(s) as control agents. For example, Harms et al. (2021) identified a novel haplotype of the alien invasive water weed *Hydrilla verticillata* (L.f.) Royle, and found that the source population was from north-eastern China. This allowed the researchers to conduct targeted herbivore guild collections from this region.

The NPNS *E. curvula* wasp that was assigned the morphospecies name ‘*Tetramesa* sp. 5’ does not appear to belong to the *Tetramesa* genus, based on the present nuclear 28S results. It is possible that the *Tetramesa* as it currently stands should be divided into two genera: one comprising the predominantly PNS groups, and the other the NPNS clades. This would need to be achieved using an integrative approach, where genetic methods are used in conjunction with expert taxonomists. Both *Tetramesa* sp. 4 and *Tetramesa* sp. 5 are currently being used in host-specificity tests on native Australian *Eragrostis* species, as this will be the ultimate deciding factor in their utility as biological control agents. *Tetramesa* sp. 4 has already been found in Australia on field-collected *E. curvula* (Sutton et al. 2023), making it vital to assess its distribution and possible non-target effects.

Sporobolus pyramidalis

The PNS *Sporobolus* wasps revealed two potential taxa, namely on *S. pyramidalis* (PNS.SPYR.1) and *S. africanus* (PNS.SAFR.1). The GMYC analysis suggested a split of the PNS.SPYR.1 clade into two taxa, but this was an artefact of geographic substructuring (these were from *Tetramesa* collections in the KwaZulu-Natal and Eastern Cape provinces). Both field-based and no-choice tests have revealed that *Tetramesa* sp. 1 (PNS.SPYR.1) is not able to utilise *S. africanus* (Sutton et al. 2021b), but the host specificity of the *S. africanus* wasps should be investigated further. The *Tetramesa* in clade PNS.SAFR.1 is a novel group that requires host specificity testing mirroring the work conducted on *Tetramesa* sp. 1 by Sutton et al. (2021b).

Sutton et al. (2021b) have already found that *Tetramesa* sp. 1 is suitably host specific and damaging to *S. pyramidalis* and *S. natalensis* in the field. Additionally, this wasp has been recently imported into quarantine in Australia for further host specificity testing.

Both the PNS and NPNS *Sporobolus* wasps (named morphospecies ‘*Tetramesa* sp. 1’ and ‘*Tetramesa* sp. 2’) have been shown to be host-specific in the field, where sp. 1 was found to be the more damaging of the two (Sutton et al. 2021b). The present phylogenetic results suggest that the NPNS *Tetramesa* sp. 2 wasp does not belong to the *Tetramesa* genus. *Tetramesa* sp. 2 was identified morphologically as belonging to the *Tetramesa* genus, but our genetic analyses suggest that they are sufficiently different to be considered a new or different genus. This highlights the need to update the taxonomy of the group.

The present genetic results suggest that the NPNS *Tetramesa* sp. 2 wasp may be able to feed on *E. curvula* (two wasp samples out of the nine that were collected on *S. pyramidalis*), but field host range surveys by Sutton et al. (2021b) have found that the wasp was specific to only *S. pyramidalis* and *S. natalensis*. Laboratory-based host specificity tests have also been conducted on over 20 non-target species, including eight native South African *Sporobolus* species, with no non-target feeding recorded to date (Guy Sutton, unpublished data).

This scenario is an example of why it is imperative to have correctly identified each prospective agent in a biological control programme, and to have a solid understanding of their life histories and interspecific interactions in order to achieve the greatest level of damage. Since *Tetramesa* sp. 2 may be able to use *E. curvula* as a marginal host in the field, and that the wasp is less damaging than *Tetramesa* sp. 1, it might be prudent to only use *Tetramesa* sp. 1 for biological control.

Hyparrhenia hirta and *Andropogon gayanus*

The NPNS *Tetramesa* collected on *Hyparrhenia hirta* and *Andropogon gayanus* that formed a sister group to the PNS clade were host-specific and formed a monophyletic clade in both gene trees. This is not surprising, since *H. hirta* and *A. gayanus* are phylogenetically closely related, both being in the tribe Andropogoneae (Skendzic et al. 2007), and

structurally similar (i.e., both are tall-statured grasses) (Canavan et al. 2019b).

The COI GMYC species delimitation results suggested that this group may comprise three cryptic species, and could be considered as potential biological control agents because they are unique and host specific. *Hyparrhenia hirta* has naturalised in the USA and many parts of Europe, but it is particularly problematic in Australia, where it has become highly invasive (Chejara et al. 2010). It has also been reported as a problematic weed in wheat fields in Pakistan (Hussain et al. 2004). Similarly, *A. gayanus* has invaded the northern regions of Australia’s tropical savannas (Rossiter-Rachor et al. 2009). Invaded regions such as these could benefit from the *Tetramesa* biological control agents identified here, particularly in mitigating intense bushfires and devalued land. Although there were only two specimens in this group, it would be worth conducting additional surveys on *A. gayanus* in Zimbabwe, as there is likely to be a large degree of species diversity on the grass. Additional field surveys on other close relatives within the Andropogoneae are also required to assess their potential as bio-control agents.

Southern African *Tetramesa* phylogenetics

The nuclear 28S rRNA marker was effective in broadly separating what are likely true *Tetramesa* from other described or undescribed genera, where a suggested sequence divergence of ~ 3 to 3.5% can be used for genus-level delimitation. Our interspecific p-distances were comparable to Chen et al. (2004), who reported interspecific distances of 1.69–13.5% for their eurytomid data set. Of the four 28S sequences identified as *Tetramesa* gleaned from GenBank, two fell within the proposed *Tetramesa* clade (sourced from Chen et al. (2004) and Munro et al. (2011)) while the other two (Gillespie et al. (2005), and a sequence from unpublished material (<https://www.ncbi.nlm.nih.gov/nuccore/DQ080114>), fell in a NPNS polytomy that was more similar to some *Eurytoma* sequences, and with a divergence of as much as 7.1% from the *T. romana* group. This high nuclear divergence suggests that these NPNS GenBank specimens were either misidentified, or that the *Tetramesa* genus needs to be split using morphological and molecular tools. Similarly, all the COI sequences deposited on BOLD that were identified as *Tetramesa*

(collected in Canada and Germany) showed sequence divergences of nearly 20% compared to *T. romana* and *T. bambusae*, which is a clear indication that these specimens likely belong to a different genus. The lack of genetic data across the entire genus is an impediment, and more sequence data is needed to revise the genus.

In agreement with Chen et al. (2004), the nuclear 28S rRNA marker is preferred as an initial identification guide, because it yielded well-supported basal nodes and broader-scale taxonomic relationships that will be useful for genus-level taxonomic revisions. The COI marker should be used as second filter to focus on specific target groups identified in the nuclear gene tree, as it provided greater resolution within the PNS group and revealed some potential cryptic species and/or genetically distinct populations. Numerous polytomies, however, were a confounding factor in inferring how these groups are evolutionarily related. Additionally, the COI phylogeny did not produce the same monophyletic *Tetramesa* group as was seen using the 28S marker, and yielded surprisingly high sequence divergence values between the Northern Hemisphere *T. romana* and *T. bambusae* groups (10.8%). It was unexpected that the NPNS *H. hirta* and *A. gyanus* and the *T. romana* clade in particular did not cluster with the PNS group in the COI phylogeny as it did in the 28S phylogeny, and that the COI genetic divergences were unusually high for these groups.

It is known that the COI marker could yield unexpected results when delimiting species that have undergone a rapid, recent radiation, as the lack of recombination in the mitochondrial genome can lead to an overestimation of sequence divergences due to the accumulation and retention of mutations (Hupalo et al. 2023). Comparatively, due to recombination, nuclear markers will thus more readily indicate renewed gene flow between previously isolated populations (e.g., due to climatic cycles and habitat changes), which can result in conflicting phylogenies (Eyer et al. 2017; Després 2019). Additionally, it is likely that these wasps can reproduce facultatively via thelytokous parthenogenesis (i.e., a form of asexual reproduction where diploid daughters are produced from unfertilised eggs) (Moran and Goolsby 2009). Compared to the default reproductive mode of arrhenotoky in the Hymenoptera (i.e., unfertilised eggs develop into haploid males and fertilised eggs

develop into diploid females), thelytoky can lead to increased levels of homozygosity over time (Mateo Leach et al. 2009). It is even possible that infection by different *Wolbachia* strains can cause reproductive isolation between intraspecific populations (e.g., in *Nasonia* wasps (Bordenstein et al. 2001)). Future phylogenetic studies could investigate a wider range of nuclear markers—both ribosomal and protein-coding—and also the relationship between *Wolbachia* and their *Tetramesa* hosts in order to determine the degree to which these endosymbionts might be affecting genetic diversity, and under what conditions the wasps change their mode of reproduction.

This study has presented the first phylogenetic analysis of southern African *Tetramesa*, and has revealed multiple taxa that are new to science. Combined with field host range data, we have identified at least five *Tetramesa* taxa that show promise as biological control agents (these were namely clades PNS.SPYR.1, PNS.SAFR.1, NPNS.HHIR.2, NPNS.HHIR.3, and NPNS.AGAYA.1 in Fig. 2 and Supplementary Fig. S6). These wasps appear to be host-specific to *S. pyramidalis*, *S. africanus*, *H. hirta*, and *A. gyanus*. Based on field host range data, *Tetramesa* sp. 4 on *E. curvula* showed evidence of oligophagy across congeners, and is unlikely to be suitably host-specific for release in Australia where there is a very large diversity of *Eragrostis* species. Recommendations for future research include: (1) incorporating multiple other nuclear regions and ddRADseq techniques into phylogenetic analyses to refine divergence thresholds for delimiting true *Tetramesa* from other eurytomid genera, as well as to resolve the high incidence of polytomies observed, (2) using phylogenetic methods in conjunction with morphological and ecological data to revise the taxonomy of the group (with the possibility of creating subgenera), and (3) conducting further host-specificity tests, impact assessments, and potential hybridisation trials with the different *Tetramesa* taxa to determine their efficacy as biological control agents.

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Declarations

Competing interests We have no competing interests to declare.

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