

Rapid Communication**First record of *Megabruchidius tonkineus* (Pic, 1904) (Coleoptera: Chrysomelidae, Bruchinae) in Australia**

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OPEN ACCESS**Abstract**

In July 2021, based on a series of photographs posted in the citizen science platform iNaturalist, the Chief Plant Health Officer Unit of Victoria was alerted to a sighting of a suspect exotic chrysomelid from the Royal Botanic Gardens (RBG), East Melbourne, Victoria, Australia. Specimens were collected and forwarded to the Crop Health Services diagnostic laboratory (Agriculture Victoria) for identification. These were identified as *Megabruchidius tonkineus* (Pic, 1904) (Chrysomelidae: Bruchinae) based on literature, reference specimens and via COI barcoding. This report presents the first record of *M. tonkineus*, which also represents the first record for the exotic genus *Megabruchidius* Borowiec, 1984 for Australia.

Key words: honey locust seed beetle, Fabaceae, *Gleditsia*, honey locust, seed beetles, detection, COI barcode, morphological identification, exotic, biosecurity

Introduction

Megabruchidius Borowiec, 1984, (Coleoptera: Chrysomelidae), belongs to the subfamily Bruchinae Latreille, 1802, also known as seed beetles, with about 1700 species worldwide (Reid and Beatson 2013). The larval stage of bruchine beetles feeds and develops obligatorily in the seeds within the pods of primarily Fabaceae hosts (Borowiec 1987; Southgate 1979). Due to the bruchine larval host specificity and endophagous behaviour, studies worldwide have identified species in this group as being either a potential threat, e.g., pests of economically important legumes or stored pulse grains (Kurtek et al. 2017; Lambrides and Imrie 2000; Yus-Ramos et al. 2014), or beneficial, e.g., biocontrol agents to control fabaceous weeds (Julien and Griffiths 1998; Julien et al. 2012; Winston et al. 2014). In Australia, the subfamily Bruchinae constitutes a small group with only 30 species in 12 genera, which comprises a mix of native and introduced species (Reid and Beatson 2013). Reid and Beatson (2013) provided a key to the genera of Bruchinae present in Australia. Of the introduced species, some are deliberate introductions, e.g., *Acanthoscelides puniceus* Johnson, 1983 and *A. quadridentatus* (Schaeffer, 1907) both introduced under biocontrol

programs in the Northern Territory to control infestations of *Mimosa pigra* L. (Wilson and Flanagan 1991), while others are accidental introductions, e.g., *A. macrophthalmus* (Schaeffer, 1907) (Reid and Beatson 2013; Winston et al. 2014).

Megabruchidius beetles have previously been recorded only from border quarantine interceptions and are not considered established in Australia (Reid and Beatson 2013), although one unverified visual record can be found on iNaturalist from Narromine, NSW dating back to 2017. *Megabruchidius* currently contains three species of Asian origin (Borowiec 1984; Tuda and Morimoto 2004) — *M. tonkineus* (Pic, 1904), *M. dorsalis* (Fåhraeus, 1839) and *M. sophorae* Tuda & Morimoto, 2004. While *M. sophorae* appears to be limited to Japan (Yus-Ramos 2009), *M. tonkineus* and *M. dorsalis*, both originally from Southeast Asia are widespread in Europe and are considered invasive by the many countries which reported the species (Sajna 2019; Šipek et al. 2022; Yus-Ramos et al. 2014). *Megabruchidius tonkineus* and *M. dorsalis* have also been found to co-exist on the same host, even in the same pods (Šipek et al. 2022), in some cases, *M. dorsalis* was observed to be the more dominant species (Horvat and Sajna 2021b).

Megabruchidius tonkineus, the honey locust seed beetle, was first described by Pic (1904) as *Laria tonkinea* from “Tonkin” (northern Vietnam) on *Gleditsia australis* Hemsl. ex. F. B. Forbes & Hemsl. (Fabaceae: Caesalpinioideae). Its Asian origin further noted by Wendt (1980) who examined some specimens collected from a hotel in Germany, which had “white seeds from an unknown *Phaseolus* species from Vietnam” stored on site. Wendt (1980) moved the species into the genus *Bruchidius* Schilsky, 1905, (as *Bruchidius tonkineus*); and four years later Borowiec (1984) established *Megabruchidius* and moved *Bruchidius* into this genus, hence the new name combination *Megabruchidius tonkineus* (Pic, 1904). After Wendt (1980), *M. tonkineus* was subsequently reported by various European countries: Hungary (Jermly and Szentesi 2002), Bulgaria (Stojanova 2007), France (Delobel and Delobel 2008), north west Caucasus (Korotyayev 2011), Slovakia (Bezdek 2021; Majzlan 2011), Switzerland (György and Germann 2012), Greece (Yus-Ramos et al. 2014), Serbia (Gagić-Serdar et al. 2014), Eastern Croatia (Kurtek et al. 2017), Spain (Yus-Ramos and Carles-Tolrá 2017), Romania (Pintilioaie et al. 2018), Montenegro (Šipek et al. 2022); and South American countries: Chile and Argentina (Yus-Ramos 2009). In all these countries, *M. tonkineus* had infested the seed pods of the American tree, honey locust, *Gleditsia triacanthos* L., which is extensively cultivated worldwide in ornamental and agroforestry settings (POWO 2022a).

This article reports on the verified record of *Megabruchidius tonkineus* found in the Royal Botanic Gardens (RBG), East Melbourne, Victoria, Australia; from a host other than *Gleditsia triacanthos*, with the discussions of some aspects requiring further studies.

Materials and methods

The identities of six specimens of *Megabruchidius tonkineus* (Pic, 1904) were verified and have been deposited in the Victorian Agricultural Insect Collection (VAIC), AgriBio Centre, Bundoora, Victoria, Australia (collection numbers: VAIC 083303–083308). Two other reference specimens of *M. tonkineus* were kindly provided by the Department of Agriculture, Water and Environment (DAWE) for comparison. These were intercepted at the Melbourne airport as part of the regular pre-border quarantine inspections, from honey locust seed pods (1 ♂) and from unidentified seeds (1 ♀), all from Vietnam. These specimens have also been deposited in the VAIC (collection numbers: VAIC 083309–083310).

The specimens were studied and imaged using a Leica M205C stereo microscope with a Leica MC190 HD camera, and the montage images (Figure 1A–F) were processed via the LAS X Life Science Microscope Software platform.

The specimens were examined and the diagnostic characters verified against the genus and species description in Borowiec 1984, 1987; Tuda and Morimoto 2004; and Yus-Ramos 2009. We observed the following combination of characters on the specimens, which identified them as *Megabruchidius tonkineus*. Briefly, characteristically blackish distally on the elytra; hind femur with a small tooth ventrally on the internal carina along with several small denticles, (the presence of the tooth and the shape of the denticles are variable between the specimens, even between the males); middle of the first ventrite of males with a circular patch; a pair of distinctive, elongate depressions on the pygidium of the females; the antennae are not sexually dimorphic. The presence of a distinctive long and robust spur (also called a mucro (Borowiec 1984)) on the apex of the hind tibia in both sexes, ruled out *M. dorsalis* and *M. sophorae* as the spur in the latter two species is significantly shorter (Tuda and Morimoto 2004).

DNA extractions were performed individually using one tarsus per specimen for five specimens following a modified Walsh et al. (1991) protocol. Briefly, 100 µL of Chelex®100 (Bio-Rad Laboratories, Hercules, CA) as 5% (w/v), 5 µL of proteinase K (Qiagen, Hilden, Germany) and two 3 mm glass beads were added to a 1.5 ml Eppendorf tube. Beetle tissue was crushed in a Mixer Mill at 30 Hz for 1 min, incubated at 50 °C for 3 hrs followed by 80 °C for 10 min and put on ice for 10 min. Samples were centrifuged for 2 min at 17 000 rpm to ensure the resin beads were not present in suspension when template DNA was taken.

We amplified a 709 bp fragment of the mitochondrial Cytochrome Oxidase I (COI) locus using LCO1490 / HCO2198 primers (Folmer et al. 1994). Polymerase chain reaction (PCR) was performed using 17.5 µl of 1× BSA (New England Biolabs), 2.5 µl of 10× ThermoPol reaction buffer (New England Biolabs), 2 µl of 2.5 µM dNTPs (Qiagen), 1.25 µl of each 10 µM

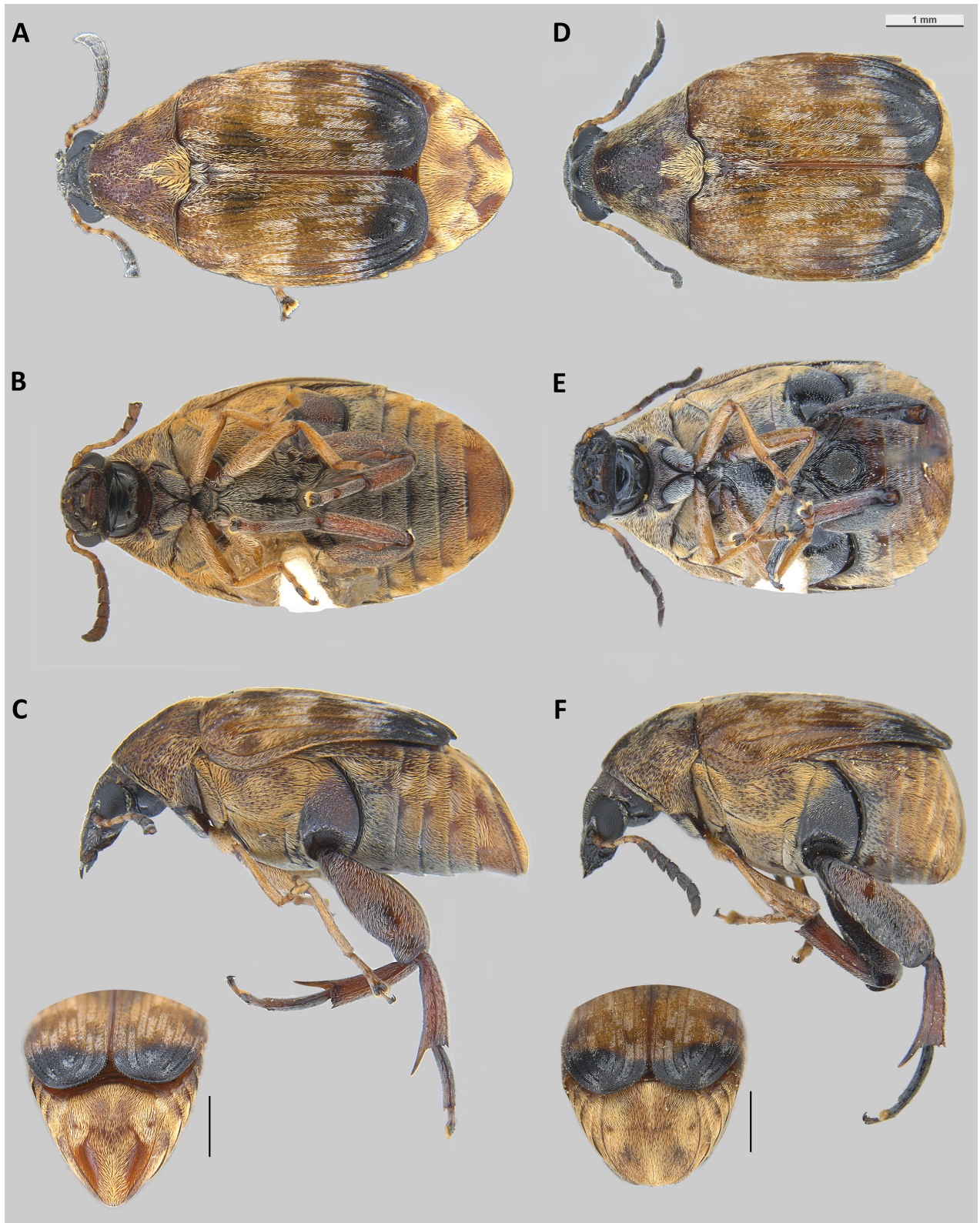


Figure 1. The dorsal, ventral, and lateral aspects of *Megabruchidius tonkineus* A–C (female), D–F (male). All figures are to the same 1 mm scale bar on the top right corner, except the posterior view of the pygidium as provided adjacent to the images (= 1 mm). Images by Lixin Eow.

primer, and 0.2 μ l (1 unit) of Taq DNA polymerase (New England Biolabs) with 2 μ l of DNA template. The PCR conditions had an initial denaturation of 2 min at 94 $^{\circ}$ C, followed by 40 cycles of denaturation at 94 $^{\circ}$ C for 30 s,

annealing at 52 °C for 45 s and extension at 72 °C for 45 s, with a final extension of 3 min at 72 °C. PCR amplification was confirmed on a 2% agarose gel. Two specimens out of five yielded sufficient DNA for successful PCR amplification. All amplified PCR products were purified and sequenced in both directions commercially by Macrogen Inc. (Seoul, Korea).

The COI sequences obtained have been submitted to GenBank (accession numbers: MZ959409–MZ959410). Searches of COI DNA sequences were conducted on BOLD (Barcode of Life Database) and GenBank (National Centre for Biotechnology Information) to determine the closest species match for each DNA sequence. The two sequences obtained in this study were 100% identical to each other and 97.7% similar to a sequence held in BOLD (GBCCH2985-19, mined from GenBank HQ177508), from an identified *Megabruchidius tonkineus* collected in Vietnam from *Gleditsia australis* (museum id Bru-Xi11).

Results

Records

Six adults, Australia, Victoria, East Melbourne: 5.viii.2021, V. Stajsic, Royal Botanic Gardens, *Gleditsia* Bed, grid CE 34, on foliage of a cultivated *Cassia* L. (possibly *C. leptophylla* Vogel), beneath the canopy of an old cultivated *Gleditsia* L. tree (leafless at the time of collection), Lat: –37.829839; Long: 144.978911. (four ♂: VAIC083305–VAIC083308; two ♀: VAIC083303–083304).

One adult, quarantine interception, Australia, Victoria, Melbourne: 27 vii 2013. ex. honey locust seed pods from Vietnam. Det. L. Watson 2013, V136795. 1 ♂: VAIC083309.

One adult, quarantine interception, Australia, Victoria, Melbourne: 21 iv 2005. ex. unidentified seed from Vietnam. Det. A. Broadley 29 xii 2011, V110066 024195VA. 1 ♀: VAIC083310.

Discussion

Host determination

The first visual record of *Megabruchidius tonkineus* adults in Melbourne dates from July 2021 from the Royal Botanic Gardens (posted on iNaturalist). Subsequently, six adults were collected in August 2021, from the foliage of a cultivated *Cassia* L. shrub, possibly *C. leptophylla* Vogel), growing underneath a *Gleditsia* L. tree which was leafless at the time of collection (austral winter). The species determination for this *Gleditsia* tree is not definitive, due to ambiguous seed pod morphology and a lack of written records of the origin for this old, cultivated tree (Val Stajsic *pers. comm.*). The tree however was closely matched to *G. × texana* Sarg. based on ITS molecular data (Gareth Holmes *pers. comm.*), which is a cross between



Figure 2. *Gleditsia* L. species pod found on the ground beneath the *Gleditsia* tree (likely *G. × texana* Sarg.) with emergence holes. Photo taken 01 October 2021 at the Royal Botanic Gardens, Melbourne by Val Stajsic.

G. triacanthos L. and *G. aquatica* Marshall (Trees and Shrubs Online 2022). Upon further examination on the ground, a number of *Gleditsia* pods were found with exit holes from which these adults may have emerged (Figure 2). This is suggestive of an earlier introduction of *M. tonkineus* into Victoria, of at least one year (assuming one generation per year). Neither *Cassia* nor *G. × texana* have previously been recorded as hosts for *M. tonkineus*.

Megabruchidius tonkineus life cycle

The life cycle of *Megabruchidius tonkineus* is not well characterised. Pintilioaie et al. (2018) proposed a multivoltine life cycle for *M. tonkineus*, with overwintering larvae, prepupae and adults, also observed by György (2007), and observed in *M. dorsalis* by Sajna (2019). Southgate (1979) discussed the wide range of Bruchinae life cycles; in summary, the life cycles of these seed beetles are intrinsically linked to climate and host phenology, in particular to pod phenology and food sources available. Melbourne has a temperate climate with mild winters which could support a multivoltine life cycle with potential hibernation phases as has been observed in European countries, however, this needs to be validated through field observations.

In general, Bruchinae adults are considered pollen feeders (Kingsolver 2004), but the adults of some species can emerge to mate and oviposit without feeding (Yates et al. 1989). In the absence of flowers or pods, the adults observed in this report would have had to feed and subsist from alternative pollen sources or survive without food for at least 5 months until *Gleditsia* flowers appear in December. We ask whether the fat reserves accumulated during the larval period would have enabled *Megabruchidius tonkineus* adults to support their energy demands for such

a long time, seeing that energy reserves accumulated as lipids during the larval stage is a critical step in bruchid development and survival (Yates et al. 1989). These observations pose interesting questions that warrant further study, to better understand the life cycle and the specific factors that affect the reproductive behaviour of this species in the south-eastern Australian climate.

Pest status and potential environmental impact

One of the concerns about the incursion of *Megabruchidius tonkineus* is whether it can become a major economic crop pest. Since Wendt (1980), who examined specimens assumed to be from “white seeds of unknown *Phaseolus* from Vietnam”, *M. tonkineus* has not been observed or reported to date anywhere in the world to feed on economically important pulse and legume crops. From this perspective this species is not considered a priority pest for Australian biosecurity agencies. This is further supported by viability experiments in laboratory conditions (see discussion in György (2007)), where *M. tonkineus* did not survive on important legumes such as *Lablab purpureus* (L.) Sweet, *Glycine max* (L.) Merr., *Lathyrus sativus* L., and *Pisum sativum* L.

Gleditsia L. (Fabaceae: Caesalpinioideae) is a genus of mostly thorny, deciduous trees, with thirteen accepted species worldwide (POWO 2022a). The likely original host of *Megabruchidius tonkineus* is *Gleditsia australis* Hemsl. ex. F. B. Forbes & Hemsl. (György and Germann 2012; Yus-Ramos 2009), a tree native to South China and Vietnam (POWO 2022a). Outside its native Southeast Asian range, in Europe and South America, *M. tonkineus* has been primarily reported from *G. triacanthos* L., a North American *Gleditsia* species widely used as an ornamental plant in gardens and urban settings throughout the world (CABI 2021; Orwa et al. 2009). *Megabruchidius tonkineus* has also been observed in Europe (completion of one generation from egg to adults) on five other *Gleditsia* species – *G. caspica* Desf., *G. delavayi* Franch, *G. ferox* Desf., *G. japonica* Miq., and *G. macracantha* Desf. (György 2007), as well as *Gymnocladus dioicus* (L.) K. Koch (Gagić-Serdar et al. 2014; György 2007); and in Australia it was observed on cultivated *Gleditsia* L. (likely *G. × texana* Sarg). It is clear *M. tonkineus* is a specialist seed feeder and has the ability to switch hosts, at least between closely related species in *Gleditsia*, which has allowed it to expand successfully outside of its native range into Europe, South America and now Australia. Furthermore, Pintilioaie et al. (2018) recorded collections of *M. tonkineus* from under the bark of plane trees (*Platanus* sp.) and in leaf litter, but the authors suggested these as overwintering locations only.

It is important to note that *Megabruchidius dorsalis* also uses the same host *Gleditsia triacanthos* L., as well as *G. japonica* Miq., *G. sinensis* Lam., and *G. rolfei* S. Vidal (Tuda and Morimoto 2004); while *M. sophorae* was

described from *Styphnolobium japonicum* (L.) Schott (Tuda and Morimoto 2004), and appears to not yet have been reported outside of Japan (György and Germann 2012), despite the tree being native to China (POWO 2022b). Neither species have been detected in Australia.

In Australia, *Gleditsia triacanthos* was first introduced in the mid-1800s both as a fodder plant and for ornamental purposes, and became widespread – found in Queensland, New South Wales, Victoria, South Australia, and Western Australia; and assessed to be an invasive plant of potential national significance in Queensland, New South Wales, and Western Australia (Centre for Invasive Species Solutions 2021). The tree species is highly adaptable, and tolerant of drought, frost, and most soil types, but prefers alluvial soil associated with riverine and floodplains (Csurhes et al. 2004; Centre for Invasive Species Solutions 2021; Victorian Resources Online 2020). *Gleditsia triacanthos* possesses long, robust thorns or spines on the trunks and branches, with thornless cultivars developed for ornamental purposes, although the latter could revert to the original spiny form with age or producing seeds that develop into the thorny form (Csurhes et al. 2004). The weedy, spiny form can cause physical harm to animals, form barriers of dense thickets around waterways preventing livestock access, and outcompete native or purpose planted vegetation, affecting both native ecosystems or agricultural lands (Centre for Invasive Species Solutions 2021). The spiny form was subjected to successful eradication in Queensland in the 1990s (Csurhes et al. 2004), and although uncommon in Victoria, does occur along waterways near and along the Murray River (Centre for Invasive Species Solutions 2021). The spineless form *G. triacanthos* L. forma *inermis* (L.) C. K. Schneid is marketed in specialty nurseries in Victoria under variety names such as “Ruby Lace”, “Elegantissima”, “Emerald Cascade”, “Shademaster”, and “Sunburst”. A number of additional *Gleditsia* species are also found in Victoria but are restricted to botanical gardens, such as the cultivated *Gleditsia* L. (likely *G. × texana* Sarg.) in the Royal Botanic Gardens, in Melbourne.

We ask whether the arrival of a specialist seed beetle such as *Megabruchidius tonkineus* could be detrimental in the long run. There is evidence that shows specialist seed beetles, in their native and introduced environments, enhance seed germination of their hosts through breaking hard seeds dormancy by larvae drilling through the seed coat, followed by well-timed larval death through different factors but particularly through drowning in water (e.g. rain or river) (Horvat and Sajna 2021a; Takakura 2002). For example, studies carried out on *M. dorsalis* have found larval seed drilling damage to be a primary prerequisite for the germination of *G. japonica* in Japan (Takakura 2002), and *G. triacanthos* in Europe (Horvat and Sajna 2021a). There is reasonable concern a similar seed predator and host interaction could occur in Australia between the introduced *M. tonkineus*

the naturalised introduced *G. triacanthos* and potentially other closely related suitable *Gleditsia* hosts. Assuming that the seed germination of *G. triacanthos*, particularly near riverine and floodplains, would be improved by larval feeding, then the arrival of this exotic beetle is concerning from the perspective of land conservation and management, as it could considerably contribute to the deterioration of important natural ecosystems, particularly for riverine, floodplains and other areas where the host plant has been declared a weed (Csurhes et al. 2004; Centre for Invasive Species Solutions 2021). Over time, costly control measures might be necessary to stop the spread of the weed, as discussed in case of the eradication operation in south-eastern Queensland (Csurhes et al. 2004).

Natural predators

Natural predators have been observed for *Megabruchidius dorsalis* in the seeds of *Gleditsia triacanthos* L. in Ukraine, with a new species *Eurytoma gleditsiae* Zerova and Fursov, 2015 (Hymenoptera, Eurytomidae) being described (Zerova and Fursov 2015). A long term field study carried out by Gagić-Serdar et al. (2014) in Serbia collected seed pods of several fabaceous tree species and recorded emerging bruchine beetles and their parasitoids. Interestingly, this study did not record any parasitoids for *M. tonkineus* from either of the hosts studied (*G. triacanthos* or *Gymnocladus dioica* (L.) K. Koch). There are many species of Eurytomidae in Australia, including species in the genus *Eurytoma* Illiger, 1807 (ALA 2022), however, whether any of the Australian endemic species in this group or other known bruchine parasitoids in Chalcidoidea or Trichogrammatidae (Pérez-Benavides et al. 2020; Van Huis et al. 1991), could parasitise *M. tonkineus*, requires further studies.

Conclusions

The presence of a reportedly highly invasive new bruchine beetle in Australia is concerning. While the introduction of *Megabruchidius tonkineus* does not seem to pose immediate risk to the economically important fabaceous crops, its environmental impact over time from its interaction with the host *Gleditsia triacanthos* needs monitoring. There is reasonable concern that *M. tonkineus* may impose an environmental cost over time particularly in areas where *G. triacanthos* is a declared weed, by promoting the germination of the hard seeds and further expanding its distribution. Specifically, the riverine areas are a concern and need to be monitored. Further research is needed to ascertain if *M. tonkineus* has become established in Melbourne outside the Royal Botanic Gardens, and whether the species is spreading to other Australian states based on the distribution of suitable hosts. Furthermore, monitoring for the presence of *M. dorsalis* in Australia is warranted given that *M. tonkineus* and *M. dorsalis* have been seen to

coexist overseas. Additionally, assessments of distribution and host compatibility studies on potential parasitoids over a complete season are an area needing further study.

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Authors' contribution

Research conceptualization: Lixin Eow, Isabel Valenzuela, Lea Rako. Sample design and methodology: Lixin Eow, Isabel Valenzuela, Lea Rako. Investigation and data collection: all authors. Data analysis and interpretation: all authors. Roles/writing – original draft, review and editing: all authors.

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