

Information supporting the application to amend the List of Specimens Suitable for Live Import to include three new species of dung beetles (Coleoptera: Scarabaeidae) as suitable for import and for release

CSIRO Health and Biosecurity



Left: Male *Euonthophagus crocatus* (from Ballerio *et al.* 2014a). Middle: Male *Onthophagus andalusicus* (from Ballerio *et al.* 2014c). Right: *Gymnopleurus sturmi* of unknown sex (from Ballerio *et al.* 2014b).

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Rationale

This proposal provides information supporting the application to amend the List of Specimens Taken to be Suitable for Live Import to include *Euonthophagus crocatus*, *Onthophagus andalusicus*, and *Gymnopleurus sturmi* as suitable for importation. This will be the first attempt at importation and release of these species of winter and spring-active dung beetles. The importation of these three species is part of the large Rural Research and Development for Profit program 'Dung Beetles as Ecosystem Engineers'.

The objective of introducing *E. crocatus*, *O. andalusicus* and *G. sturmi* is to enhance dung burial and bush fly control in the major sheep, beef and milk producing areas of southern Australia to fill the current seasonal and geographical gaps. Although many of the 23 species of exotic dung beetle already established in Australia are regarded as being spring-active, few emerge early enough to have a significant influence on the rapid increase in fly populations that commonly occurs during September and October. Southwestern Western Australia is a region where flies migrating from the northern winter, breed in the wet spring dung of 20 M sheep without dung beetles leading to the worst bushfly densities in Australia. Using climate matching software, we have identified three European species of scarabaeine dung beetle, *E. crocatus*, *O. andalusicus* and *G. sturmi* which have the potential to fill these gaps in beetle activity.

Adult *E. crocatus*, *O. andalusicus* and *G. sturmi* will be collected from several localities in south-western Europe and northern Africa and air-freighted to Australia (up to 500 pairs per consignment). The beetles will be handled similarly to the previous projects in the early 1990s and 2011-2015. Briefly, in-coming beetles will be held in an Approved Arrangement site (quarantine) in Canberra. Their eggs will be treated with disinfecting agent (Virkon®), removed from quarantine, reared to adulthood and released into the wild and/or used for the establishment of mass-rearing colonies, the progeny of which will be released. Release sites will be chosen by selecting climatically optimal sites on properties whose owners are committed to doing everything necessary to maximise the beetles' establishment, such as avoiding the use of parasiticides. Beetles will be released when they are sexually mature and physiologically synchronised with the local season. Release numbers will vary according to the numbers reared, but at any given site the aim will be to release a minimum of 500 male-female pairs of each species.

1. Taxonomy of the species

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera,

Family: Scarabaeidae

Subfamily: Scarabaeinae

Tribe: Onthophagini

Genus: *Euonthophagus*

Species: *Euonthophagus crocatus* (Mulsant & Godart, 1870)

Genus: *Onthophagus*

Species: *Onthophagus andalusicus* (Waltl, 1835)

Synonym: *Onthophagus marginalis* spp. *andalusicus*
(as reported in Boucher 1990)

Tribe: Gymnopleurini

Genus: *Gymnopleurus*

Species: *Gymnopleurus sturmi* (MacLeay, 1821)

The proposed specimens for import are *E. crocatus*, *O. andalusicus* and *G. sturmi*.

No common names for *E. crocatus*, *O. andalusicus* and *G. sturmi* were found in literature. The *Scarabaeinae* subfamily are commonly known as the dung beetles.

E. crocatus, *O. andalusicus* and *G. sturmi* are not genetically-modified organisms.

2. Information on the status of the species under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

None of the three species are listed on the CITES checklist.

Euonthophagus crocatus and *O. andalusicus* are listed on the IUCN Red List of Threatened Species as of Least Concern. *Euonthophagus crocatus* has stable populations in Morocco and is not considered likely to be declining in population quickly enough to qualify for listing in a threatened category (Ruiz *et al.* 2015). *Onthophagus andalusicus* has a wide distribution, is presumed to have a large population overall, and is not considered likely to be declining quickly enough to qualify for listing in a threatened category (Verdú *et al.* 2015).

Gymnopleurus sturmi is listed as Near Threatened on the IUCN Red List under criteria B2. This is due to its small area of occupancy, a continuing decline in habitat and number of mature individuals, and the fragmentation of its subpopulations in Europe. This species appears to have declined in presence and abundance in Western Europe (Spain, Portugal, France and Italy) over the past 30 years. It remains very abundant in northern Africa (Lumaret *et al.* 2015). For this reason, only individuals from northern Africa will be collected for

3. Information about the ecology of the species

3.1: Lifespan of the species

The exact lifespan of *E. crocatus*, *O. andalusicus*, and *G. sturmi* is unknown, but can be assumed based on existing information on the life history of these species. CSIRO will be able to gather more information on the lifespan of these species during the rearing process.

The lifespan of *E. crocatus*, *O. andalusicus* and *G. sturmi* can be assumed to be at least one year based on their life history. The active period for each species occurs once a year (section 3.13), and all three species are believed to be univoltine, i.e. one generation born per year (section 4).

3.2: Size and weight range

Euonthophagus crocatus adults are 6-12mm long (Baraud 1992), with a dry (dead specimen) weight of 22.1 mg (Errouissi *et al.* 2004).

Onthophagus andalusicus adults are 6-12mm long (Baraud 1992), with a dry weight of 31.7mg (Errouissi *et al.* 2004).

Gymnopleurus sturmi adults are 10-15mm long (Baraud 1992), with a dry weight of 85 mg (Errouissi *et al.* 2004).

3.3: Identification

The eggs and larvae of Scarabaeinae species generally look similar, as seen in Halffter and Edmonds (1982). Images of the egg of *Bubas bubalus* (figure 1) and the larvae of *O. andalusicus* (figure 2) are included as representative examples. Dung beetle eggs and larvae develop in a brood ball (Cambefort and Hanski 1991).



Figure 1: Egg of *Bubas bubalus* in brood ball (from Wright *et al.* 2015).

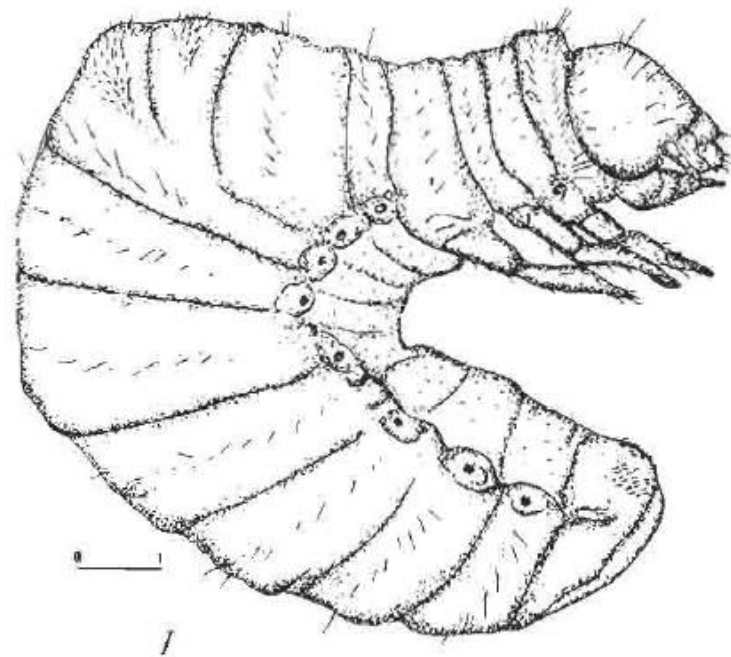


Figure 2: Larvae of *Onthophagus andalusicus* (from Barbero and Palestini 1996).

The pupa of different dung beetle species tend to look different, but the overall design is similar (Halffter and Edmonds 1982 diagrams). Diagrams of the pupa of female (figure 3) and male (figure 4) *O. andalusicus* are included as an example. The pupa develops in the brood ball, usually in a pupation chamber made from its own excrement (Cambefort and Hanski 1991).

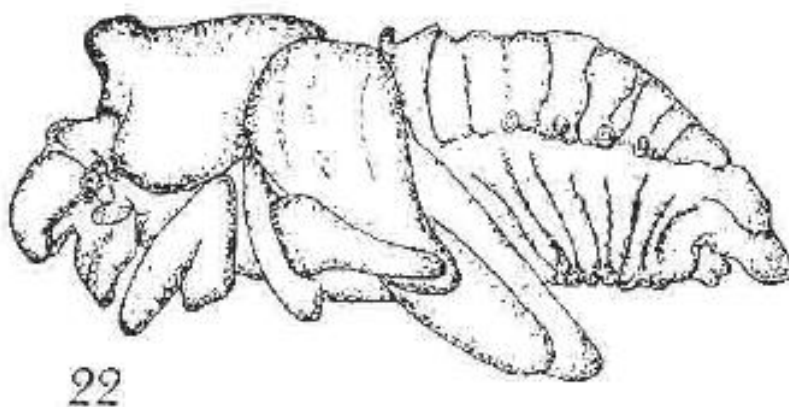


Figure 3: Female pupa of *Onthophagus andalusicus* (from Barbero and Palestini 1996).

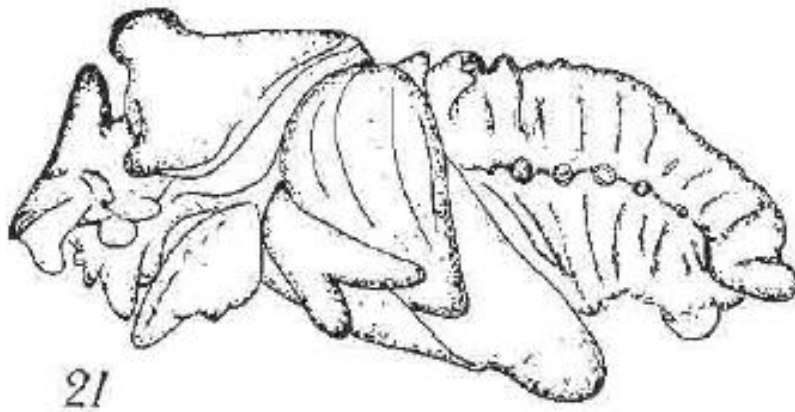


Figure 4: Male pupa of *Onthophagus andalusicus* (from Barbero and Palestini 1996).

Euonthophagus crocatus shows sexual dimorphism. Beetles of both sexes have a black body with a matte finish. Their elytra are not deeply striated (figures 5 and 6). Males have a convex pronotum with a small ridge on the side closest to the head. The male's frons are elongated with a point at each end (figure 6) (Baraud 1992). Individuals can be sexed by examining their pronotum and head.



Figure 5: Adult female *Euonthophagus crocatus* (from Ballerio *et al.* 2014a).



Figure 6: Adult male *Euonthophagus crocatus* (from Ballerio *et al.* 2014a).

Onthophagus andalusicus shows sexual dimorphism. Both sexes have a black head and pronotum. The elytra are yellow with irregular black marks (figures 7 and 8). Males have a triangular horn on top of their head (figure 8) (Baraud 1992). Individuals can be sexed by checking the head for a horn, or by checking for the differences in pygidium shape shown in figure 9.



Figure 7: Adult female *Onthophagus andalusicus* (from Ballerio *et al.* 2014c).



Figure 8: Adult male *Onthophagus andalusicus* (from Ballerio *et al.* 2014c).

Onthophagus beetles can generally be sexed by examining the final segment of their abdomen, the pygidium. This segment covers the beetle's genitals. In female dung beetles it is uniform in width, and in male beetles it becomes more narrow in the middle (figure 9) (Dung beetle UK Mapping Project 2017).



Figure 9: Unknown specimen of *Onthophagus* showing the difference in the pygidium of female and male beetles (From Dung beetle UK Mapping Project 2017).

Sexual dimorphism is not observed between male and female *G. sturmi*. Individuals are quite shiny, black in colour, and may sometimes have a blueish tinge. Their elytra are mostly smooth (figure 10) (Baraud 1992). Individuals cannot be sexed as they appear identical, but pairs can be isolated from other individuals when rolling dung balls together (Professor Jean-Pierre Lumaret pers. comm.).



Figure 10: Adult *Gymnopleurus sturmi*, unknown sex (from Ballerio *et al.* 2014b).

3.4: The natural geographic range

Euonthophagus crocatus is found in the southern parts of the Mediterranean basin, specifically northern Africa (Morocco, Algeria, Tunisia and Libya). It is not found in the Saharan regions. There have been reports of this species in southern Italy (Sicily and Calabria) and southern Spain, but these remain unconfirmed (Ruiz *et al.* 2015).

Euonthophagus crocatus is abundant in Morocco (Tauzin 1990, Janati-Idrissi 2000, Romero-Samper 2008, Lobo *et al.* 2010 cited in Ruiz *et al.* 2015).

Onthophagus andalusicus is distributed throughout the western area of the Mediterranean Basin. It is found in northern Africa (Morocco, Algeria and Tunisia), except in the Saharan regions, and in south-western Europe (Portugal, Spain, Italy and Malta) (Verdú *et al.* 2015).

Onthophagus andalusicus is abundant in Morocco, Spain, and northern Tunisia (Kocher 1958, Baraud 1985, Tauzin 1990, Romero-Samper and Lobo 2008, Verdú *et al.* 2015).

Gymnopleurus sturmi is widely distributed throughout the Mediterranean Basin. It has been recorded in Algeria, Croatia, Egypt, France, Corsica, Iraq, Israel, Italy, Sardinia, Sicily, Jordan, Lebanon, Morocco, occupied Palestinian Territory, Portugal, Saudi Arabia, Spain, the Balearic Islands, the Syrian Arab Republic and Tunisia (Lumaret *et al.* 2015). A decline in presence and abundance has been observed in France, Italy, Spain and Portugal during the past 30 years, hence its inclusion on the IUCN Red List as a Near Threatened

species. *Gymnopleurus sturmi* is observed to be very abundant in northern African dung beetle assemblages (Ruiz et al. 1995; Errouissi et al. 2004; Haloti et al. 2006; Lumaret et al. 2015).

Population limiting factors in the natural range of *E. crocatus*, *O. andalusicus* and *G. sturmi* include resource availability and competition with other dung beetle species. Dung beetle species occupying the same niche will compete for resources such as dung and space (Hanski and Cambefort 1991a). The impact of predation is unclear (see section 3.11).

With a preference for open habitats and cattle and sheep dung *E. crocatus*, *O. andalusicus*, and *G. sturmi* have not lacked a suitable niche as the Mediterranean basin was traditionally a site of heavy livestock farming (Lumaret and Kirk 1991). Changes in land use, such as the decline in livestock farming in favour of intensive agriculture, are believed to be responsible for local extinctions of *G. sturmi* observed in Spain, Portugal, Italy and France (Lumaret et al. 2015). It is possible that the use of veterinary products (parasiticides) in livestock has contributed to this decline, given the mortality risk for larvae when adults use dung contaminated with these products (Carpaneto et al. 2007 and Lumaret et al. 2012 cited in Lumaret et al. 2015).

Similar land use changes are also a threat to *E. crocatus* (Ruiz et al. 2015), while the growth of cities may cause local declines in *O. andalusicus* (Fattorini 2011 cited in Verdú et al. 2015). The larvae of both species is considered vulnerable to the use of dung contaminated with veterinary chemicals by adults (Lumaret et al. 2012 cited in Ruiz et al. 2015; Lumaret et al. 2012 cited in Verdú et al. 2015).

Dung is both eaten and used in nest construction. Dung pats can host and feed large numbers of dung beetles (Hanski 1991b; Doube and Marshall 2014), although the number of dung beetles in an area will of course be limited by the available dung. Roller beetles such as *G. sturmi* appear to have an advantage in dung collection over tunnelling beetles such as *E. crocatus* and *O. andalusicus*, as roller beetles do not have to construct their burrow before collecting dung (Hanski and Cambefort 1991a).

For tunnelling species such as *O. andalusicus* and *E. crocatus*, at high population densities competition for space under dung pats can become so intense that breeding is reduced or prevented. This may not be an issue in an established colony as female dung beetles only need to produce two young per year to maintain colony size (Doube and Marshall 2014). *Gymnopleurus* species avoid competition for space by rolling their dung ball away from the dung pat after collection. Ball stealing behaviour is observed in the *Gymnopleurus* genus, between individuals of the same species and of different roller species (Hanski and Cambefort 1991a).

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are found in assemblages of other pasture-dwelling dung beetle species in their native range (Janati-Idrissi *et al.* 1999; Errouissi *et al.* 2004; Haloti *et al.* 2006; Romero-Samper and Lobo 2008; Labidi *et al.* 2012). These species clearly inhabit the same niche and do not appear to severely limit each other's activity.

3.5: Is the species migratory?

Dung beetles are not known to be migratory, but may disperse to find resources (i.e. dung). Diapause is sometimes used to avoid unfavourable seasonal conditions (section 3.6). Within its native habitat *O. andalusicus* is classed as a resident species (Verdú *et al.* 2015), confirming that this species is not migratory.

3.6: Does the species have the ability to hibernate in winter or aestivate in summer?

Many species of dung beetle engage in diapause at different times during their life history (Hanski and Cambefort 1991 various pages), including species within the *Onthophagus* and *Gymnopleurus* genera (Cambefort 1991b; Lumaret and Kirk 1991). Diapause can occur as an evolutionary adaptation to tolerate seasonal conditions (Edwards 1982; Hanski and Cambefort 1991b), and is sometimes required before breeding can begin (Lumaret and Kirk 1991; Wright *et al.* 2015).

Diapause can be expected for all three species due to their life history. *Euonthophagus crocatus*, *O. andalusicus* and *G. sturmi* are univoltine species (section 4.3). They breed once a year and have limited activity during the colder months (section 3.13). These species presumably engage in diapause during the months that they are not abundant, hence their absence from the soil surface.

Based on experiments in rearing *O. andalusicus* CSIRO has determined that this species requires an over-winter diapause period to breed. This period occurs following the maturation feeding of young adult beetles. *Euonthophagus crocatus* and *G. sturmi* are believed to require an overwintering period as well, but more study is needed to be certain (Professor Jean-Pierre Lumaret pers. comm.).

3.7: Habitat requirements

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are distributed around the Mediterranean basin (south-western Europe and northern Africa). They are found in winter rainfall and arid climate areas (Houston 1982). This is reflected in environmental niche modelling of these species (section 7.3, figures 13, 14, 15), which indicates that they would be suited to the equivalent climate areas in Australia (Houston 1982). The winter rainfall climate type of the Mediterranean has hot, dry summers and cool, wet winters (Houston 1982). Arid climates have low precipitation and are warm or hot throughout the year (Arnfield 2018).

Euonthophagus crocatus prefers open habitats and humid pastures but is sometimes found in forested areas (Romero-Samper and Lobo 2008; Labidi *et al.* 2017). *Gymnopleurus sturmi* prefers open, dry sites, including grasslands, shrublands, sandy beaches, and agricultural sites (Verdu 1998, Carpaneto *et al.* 2011, Lumaret 1990, Sanchez piñero pers. obs. 2014 cited in Lumaret *et al.* 2015). It prefers clay or sandy soil (Lumaret 1990, Sanchez piñero pers. obs. 2014 cited in Lumaret *et al.* 2015). *Onthophagus andalusicus* prefers wetter habitats in dry, hot areas of the Mediterranean, such as lagoons, salt marshes, coastal areas, and humid pastures (Martín-Piera 1984, Verdú 1998, Dewhurst 1979 and 1980 cited in Verdú *et al.* 2015; Labidi *et al.* 2017).

All three species nest in soil beneath or near dung pats. Deeper nests are provisioned with dung and used for laying eggs, and more shallow nests are used for the maturation feeding period of immature adults (Doubé and Marshall 2014). *Euonthophagus crocatus* and *O. andalusicus* are tunneler dung beetles, meaning that they tunnel directly into the soil beneath livestock dung to form nests. *Gymnopleurus sturmi* is a roller dung beetle, meaning that it removes dung from the pat, forms it into a ball, and rolls it away to bury just below the soil surface (Halffter and Edmonds 1982).

Information on the potential for these species to utilise habitat near waterways can be found in section 8.

3.8: Diet, including potential to feed on agricultural plants

Adult dung beetles live off the juices of moist dung. Larvae eat the dried out, solid dung contained in their brood ball (Doubé and Marshall 2014).

Euonthophagus crocatus prefers cattle and sheep dung, but will also eat horse dung, and occasionally goat dung or human faeces (Janati-Idrissi 2000; Romero-Samper 2008; Chamorro 2013). *Onthophagus andalusicus* prefers cattle and sheep dung (Errouissi *et al.* 2004; Errouissi *et al.* 2009). *Gymnopleurus sturmi* prefers sheep dung, but will also eat cattle dung, horse dung, and human and dog faeces (Errouissi *et al.* 2004; Lumaret *et al.* 2015).

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* will benefit from the abundance of their preferred dung types, as Australia has many sheep and cattle distributed through southern Australian pasture. The national herd of cattle stands at about 26 million animals (Australian Bureau of Statistics 2018), distributed as shown in figure 11. The national herd of sheep stands at about 72 million animals (Australian Bureau of Statistics 2018), distributed as shown in figure 12.

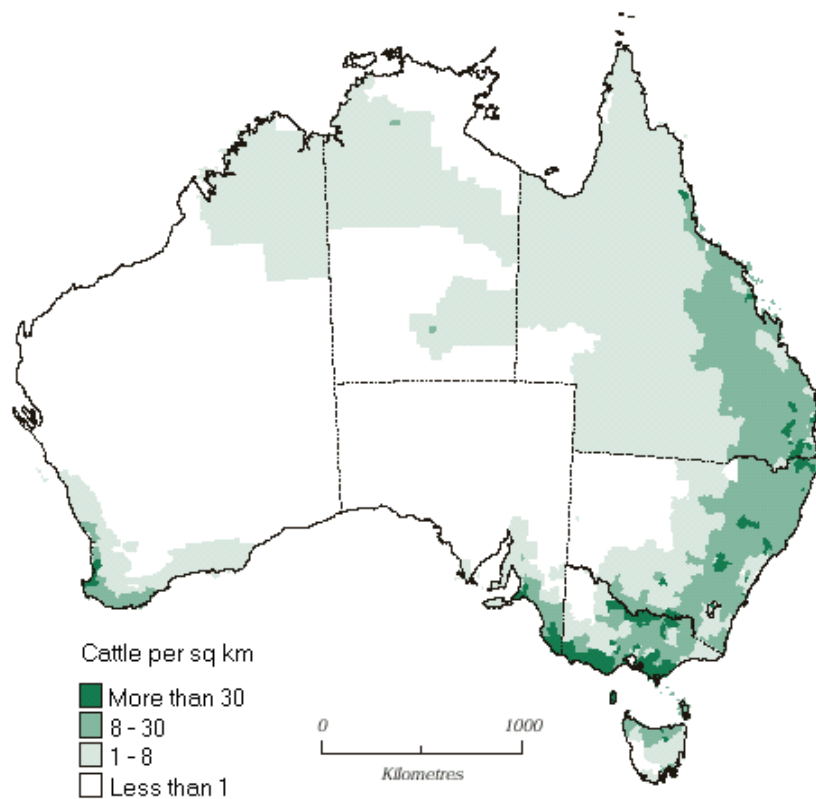


Figure 11: Distribution of cattle in Australia (Australian Bureau of Statistics 2003).

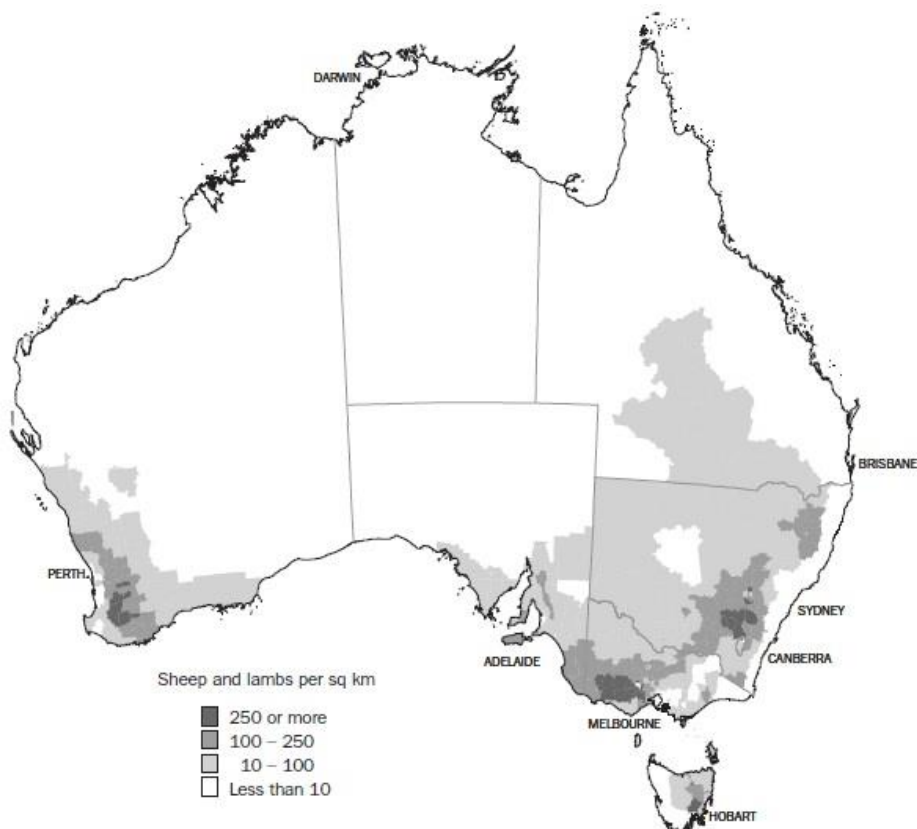


Figure 12: Distribution of sheep in Australia (Australian Bureau of Statistics 2003).

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not likely to thrive outside the areas outlined using environmental niche modelling due to the climate being unsuitable (section 7.3), so will not take complete advantage of the wide distribution of sheep and cattle in Australia.

As these species only consume dung, they will not feed on any agricultural plants.

3.9: Social behaviour and groupings

Dung beetles are found in groups when feeding on or collecting dung from dung pads (Hanski and Cambefort 1991a; Doube and Marshall 2014). Large numbers may be found on a single pad (Hanski 1991b; Doube and Marshall 2014). Dung beetles are not social (Hanski 1991b), but breeding pairs often co-operate during nesting (Halffter and Edmonds 1982). For example, male-female pairs of *Gymnopleurus* beetles will work together to move the nesting ball and bury it in the soil. The male leaves after copulation, leaving the female to shape the brood ball and lay eggs (Cambefort and Hanski 1991; Lumaret *et al.* 2015). *Onthophagus* and *Euonthophagus* species engage in similar co-operative behaviour during nest-building (Halffter and Edmonds 1982).

3.10: Territorial and aggressive behaviours

Tunnelling dung beetles such as *E. crocatus* and *O. andalusicus* are thought to mark their nests with pheromones to repel other dung beetles, so that nests under the pat usually remain well spaced out (Cambefort and Hanski 1991).

Male dung beetles may fight each other for mates (Knell 2011). Little species-specific information on mate competition between *E. crocatus*, *O. andalusicus* and *G. sturmi* males is available. It is difficult to make assumptions about mate competition behaviour in these species based on observations of other species. For example, horned dung beetles use their horns to fight, but beetles without horns may still fight by head-butting or grappling with each other (Knell 2011). So it cannot be assumed that horned *O. andalusicus* males fight, while hornless *E. crocatus* and *G. sturmi* males do not. It is safest to assume that males fight when rearing these species. CSIRO makes allowances for mate competition by providing equal numbers of males and females during breeding (see section 11).

Dung beetles do not pose a bite risk for humans due to the shape of their mouth parts (section 3.12).

3.11: Natural predators

Predators found in the natural range of *E. crocatus*, *G. sturmi* and *O. andalusicus* include birds such as owls, buzzards, and larks, and mammals such as badgers and small rodents (Young 2015). Predation on dung beetles is usually thought to be uncommon (Young 2015)

and is not considered to be a limiting factor to established colonies in Australia (Doubé and Marshall 2014). It is worth noting that the decline in numbers of *G. sturmi* is believed to be due to land use changes rather than predation (Lumaret *et al.* 2015). However, in a global review Young (2015) argues that predation is more common than previously claimed, and that birds are the most significant group of predators. Overall, the extent of predation as a limiting factor of dung beetle populations is unclear, and further research is suggested by Hanski (Hanski 1991a).

Within southern Australia, native and introduced dung beetles are preyed upon by bird species such as magpies, ravens, kookaburras and ibis, and mammals such as foxes and bats (Doubé and Marshall 2014; Young 2015). Doubé and Marshall (2014) acknowledge that predation affects dung beetles introduced to Australia, and that birds in particular can consume large numbers of the beetles. However, they argue that predation should not eliminate established colonies due to paddocks being capable of hosting large numbers of beetles (up to 1 million beetles can be present in a paddock containing 50 cattle) and adult beetles breeding in deep tunnels being out of the reach of predators. Colonies of introduced dung beetles have been observed to persist even under heavy predation by ibis, bats and foxes (Doubé and Marshall 2014).

Although the offspring of *E. crocatus*, *O. andalusicus* and *G. sturmi* will benefit from the protection of their brood mass or brood ball within the nest, they will likely not be cared for by their parents (section 4.2). Species that do not care for their brood balls leave them vulnerable to disease and parasites (e.g. fungi) (Simmons and Ridsdill-Smith 2011). The unprotected young are also vulnerable to soil invertebrates (Simmons and Ridsdill-Smith 2011), although there are not believed to be any invertebrate predators of dung beetles in Australia (Doubé and Marshall 2014).

3.12: Characteristics that may cause harm to humans and other species.

Dung beetles are not capable of biting humans or other animals due to structure of their mouth parts. They consume dung juices, and so have mouth parts designed for sucking up liquid and grinding nutritious particles (Cambefort 1991c).

As *E. crocatus*, *G. sturmi*, and *O. andalusicus* utilise cattle and sheep dung in open habitats they are not expected to pose a threat to local dung beetle fauna, which is adapted to marsupial dung in forest or woodland habitats (Doubé *et al.* 1991). This is supported by the observation that similar numbers of native dung beetles have been caught before and after the introduction of foreign dung beetles (Tyndale-Biscoe and Vogt 1996).

Some native dung beetle species of southern Australia (e.g. *Onthophagus australis*, *Onthophagus ferox*, *Onthophagus mnischei*) are occasionally found in cattle dung (Doubé *et al.* 1991). Reports of potential competitive exclusion was only found in reference to *O. ferox* at a site in WA (Ridsdill-Smith and Hall 1984, Dadour pers. comm. cited in Ridsdill-Smith

and Edwards 2011). However, this was based on an observation rather than an experimental setting or ongoing study, and although a decrease was observed it may not have been solely caused by introduced dung beetle species. *Onthophagus ferox* and other native species found in livestock dung should persist as they can continue to utilise marsupial dung in woodland habitats.

Information on the risk of dung beetles carrying parasites or diseases and mitigation measures are described in section 8.2 and section 11.

3.13: Active period

Activity data for these species was mostly taken from Moroccan studies, which seem to be the most common source of information on the active periods of *E. crocatus*, *O. andalusicus* and *G. sturmi*. This information can be considered relevant because co-ordinate data from Morocco was used in environmental niche modelling for all three species, and CSIRO plans to collect these species from localities in Morocco.

During their active period adult dung beetles engage in breeding behaviour. *Euonthophagus crocatus*, *O. andalusicus* and *G. sturmi* breed multiple times during their active period, as is typical for beetles in their tribe or breeding pattern (section 4.2). The active period of *E. crocatus* in Morocco varies depending on the region. It is active during winter and spring in most of the country, with a maximum abundance from mid-spring to early summer (April-June) (Romero-Samper 2008, Janati-Idrissi 2000, Kadiri 1989, Haloti et al. 2006 cited in Ruiz et al. 2015). In the north-west region of Ceuta it is active during spring and summer, with a maximum in early to mid-spring (March-April) (Ruiz et al. 2015). *Onthophagus andalusicus* is active from late winter to late summer (February-August) in Morocco. It is abundant from late winter to late summer (February-June), but seems to be most abundant during spring (Janati-Idrissi 2000, Romero-Samper and Lobo 2008 cited in Verdú et al. 2015). *Gymnopleurus sturmi* is active from late winter to late summer (February-August) in Morocco, with a maximum abundance in mid to late spring (April-May) (Janati-Idrissi 2000).

The active seasons of different populations of these species may vary depending on elevation. For example, *E. crocatus* populations in high-altitude areas of Morocco tend to be active in winter and spring, whereas populations in the low-lying Ceuta region are active in spring and summer (Ruiz et al. 2015). Although French data was not included due to a lack of relevance, it has been observed that *G. sturmi* populations in low-lying areas of France tend to be active from late spring to early autumn (Lumaret 1990), whereas populations in Morocco, a largely mountainous country, are mostly active from late winter to late summer (Janati-Idrissi 2000).

The active seasons of these species can likely be extrapolated to Australia due to the similarity in the climate of their native range and climate of their predicted distribution range (see section 3.7 and section 7.3, figures 13, 14, 15). The activity period of *E. crocatus* and *G.*

sturmi populations in south-western Australia may also vary depending on the elevation of population habitats.

4. Information on the reproductive biology of the species

Dung beetle breeding and nesting behaviour can be broadly divided into different breeding patterns, which different subfamilies, genera, and species fall into. For example, the specifics of rolling and tunnelling behaviour vary and so are classed into different patterns or types (Halffter and Edmonds 1982; Cambefort and Hanski 1991). Species within each tribe or genus also tend to share specific behaviours, e.g. *Onthophagus* females will generally lay more eggs than *Gymnopleurus* females (Cambefort and Hanski 1991).

Literature on the reproductive biology of *E. crocatus*, *O. andalusicus* and *G. sturmi* is scarce. CSIRO facilities in Europe and Morocco are currently breeding colonies of *E. crocatus*, *O. andalusicus* and *G. sturmi* to gain specific information in their reproductive capabilities, in anticipation of their role in the dung beetle project. The reproductive behaviour of these species will be assumed based on a combination of the typical behaviour of similar species and the discoveries made by CSIRO.

4.1: The age at maturity

The exact age of *E. crocatus*, *O. andalusicus* and *G. sturmi* at maturity is unknown. All dung beetle species can begin breeding after their maturation feeding, which occurs just after the adult beetle emerges from their brood chamber (Doubé and Marshall 2014). Due to limited food contained in the brood mass or brood ball young adult beetles have low body fat and immature gonads. The maturation feeding period allows dung beetles to put on weight and finish maturing their ovaries or testes (Cambefort and Hanski 1991; Doubé and Marshall 2014). After emerging, the young beetle searches for fresh dung to feed on. Tunneler species such as *E. crocatus* and *O. andalusicus* excavate shallow tunnels under the pat in which they feed on the juices of buried dung (Doubé and Marshall 2014). *Gymnopleurus* species such as *G. sturmi* engage in maturation feeding at the dung pat (Cambefort and Hanski 1991).

4.2: The species' ability to reproduce

Dung beetles are usually ready to reproduce following their maturation feeding period (Doubé and Marshall 2014). However, some Mediterranean dung beetle species require a period of diapause prior to breeding (Lumaret and Kirk 1991). CSIRO has observed that *O. andalusicus* requires an over-winter diapause period to breed, and suspects that this will be the case for *E. crocatus* and *G. sturmi* as well.

Temperature and rainfall are both considered triggers for seasonal activity of Mediterranean climate beetles. The extent to which these factors trigger the activity of *E. crocatus*, *O. andalusicus* and *G. sturmi*, and is unclear as the three species have slightly different activity

patterns to most other Mediterranean climate beetles (Lumaret and Kirk 1991), becoming active earlier in the year and remaining active until late summer (section 3.13). The three species may have a slightly varied seasonality to avoid competition, as is the case with *Gymnopleurus* species in West African savannahs (Hanski and Cambefort 1991b).

Seasonal dung quality appears to be a factor in the breeding activity of dung beetle species in Australia (Doubé *et al.* 1991). Studies on dung beetles introduced to south-western Australia indicate that dung quality effects the breeding success of some species, with the largest brood sizes seen in spring dung and the smallest in summer dung (Doubé *et al.* 1991). Cattle dung quality in the Mediterranean climate of south-western Australia varies depending on pasture production, which is highest in spring and lowest in summer (Ridsdill-Smith 1982). It is possible that dung quality is already an influence on the breeding activity of *E. crocatus*, *O. andalusicus* and *G. sturmi* in their native area due to their abundance reaching its highest levels during spring (section 3.13). High quality dung has also been found to encourage bush fly abundance (Hughes 1970), as observed in the proliferation of flies in south-western Australia during the spring (Matthiessen 1982).

Scarabaeidae beetles meet at the dung pat. They are believed to identify potential mates using pheromone signalling (Cambefort and Hanski 1991). Onthophagini tribe dung beetles have two ways of beginning the nesting sequence. Either the pair will mate then build their nest, or the female will begin building a nest independently, attracting a male to mate with. The male and female provision the nest with dung, forming a brood mass. The female hollows out chambers throughout the mass, in which she lays her eggs. After sealing up the brood chambers the female abandons her nest (Cambefort and Hanski 1991). Female Onthophagini beetles typically construct multiple nests during their active season (Halffter and Edmonds 1982).

Gymnopleurus females begin the mating sequence by constructing a dung ball at the dung pat, which is used as a sexual display to attract males. The male rolls the dung ball while the female shapes it. Dung beetles of the same or of other species may attempt to steal the nesting ball from the pair. Male beetles usually engage in ball-stealing behaviour. After moving away from the dung pat the pair bury their dung ball a few centimetres below the soil surface. Mating usually occurs within this shallow burrow. The male leaves after mating, at which point the female shapes the dung ball into one or two pear-shaped brood balls. She lays an egg in the narrow end of each pear. After sealing up the brood ball the female abandons her nest (Cambefort and Hanski 1991). Ball-rolling species typically build multiple nests throughout their active season (Halffter and Edmonds 1982).

Female dung beetles have been observed defecating on the brood material during its preparation, probably to encourage the development of digestive flora in larvae (Cambefort and Hanski 1991).

4.3: How frequently breeding occurs

Females of the Onthophagini tribe usually make multiple nests (Halffter and Edmonds 1982). Due to their suspected lifespan of one year (Section 3.1) and limited active season (section 3.13) *Euonthophagus crocatus* and *O. andalusicus* are univoltine species, i.e. they only produce one generation per year, but still have multiple broods and will breed throughout their active season. The univoltine breeding pattern is also observed in other *Onthophagus* species (Doubé *et al.* 1991).

Most ball rolling females will make multiple nests during the active season, taking several days between each nest to mature their eggs (Halffter and Edmonds 1982). Due to its suspected lifespan of one year (Section 3.1) and limited active season (section 3.13) *G. sturmi* is also defined as a univoltine species, as other *Gymnopleurus* species (Cambefort 1991b). Regardless of breeding frequency, the number of broods produced by the three species will be limited by their active season (section 3.13).

4.4: Are individuals singled sex or hermaphroditic?

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are single sexed (Baraud 1992).

4.5: If the female can store sperm

Female dung beetles have a sperm storing organ called a spermatheca. The retained sperm is used to fertilise eggs (Halffter and Edmonds 1982). However, female dung beetles only have one ovary and a single ovariole, usually containing only a few immature eggs (Halffter and Edmonds 1982). Because of this most dung beetle species tend to have low fecundity (Halffter and Edmonds 1982; Cambefort and Hanski 1991), although Onthophagini beetles are usually more fecund than *Gymnopleurini* beetles (Cambefort and Hanski 1991).

4.6: Number of eggs or live-born young produced at each breeding event

The number of eggs per breeding event laid by *E. crocatus* and *O. andalusicus* is unknown, but is currently being studied at the CSIRO European Laboratory. The Onthophagini tribe shows varying levels of fecundity, from one egg to many (Cambefort and Hanski 1991). One egg is laid per brood chamber as this is the typical behaviour for this tribe (Halffter and Edmonds 1982). *Onthophagus* species previously introduced to Australia lay several eggs per nest (Edwards *et al.* 2015). *Gymnopleurus sturmi* will probably lay 1 or 2 eggs per breeding event with one egg in each brood ball, as is typical for the genus (Cambefort and Hanski 1991).

4.7: If the species has hybridised with other species or has the potential to hybridise with any other species

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* have not been observed to breed hybrid individuals. Separate species of dung beetles do not usually breed hybrids due to

variance in their genital shape and size. Dung beetles must have compatible genitals to mate with each other (House and Simmons 2003; Pizzo *et al.* 2006; Robson 2008).

5. Information on whether this species has established feral populations

Euonthophagus crocatus, *O. andalusicus*, and *G. sturmi* are not known to have been introduced outside their native range, and no feral populations are known (Lumaret *et al.* 2015; Ruiz *et al.* 2015; Verdú *et al.* 2015).

6. Environmental risk assessments of the species

As far as we know, no environmental risk assessments have been carried out in Australia or overseas on *E. crocatus*, *O. andalusicus* or *G. sturmi*. The IUCN has carried out assessment on their conservation value. *Euonthophagus crocatus* and *O. andalusicus* are considered of Least Concern due to their numbers remaining stable, while *G. sturmi* is considered Near Threatened due to a decline in distribution and abundance in Europe, but not in northern Africa (Section 2).

Introduced dung beetles are considered beneficial insects in Australia. To date 45 species have been released, 23 of which have established populations (Edwards 2007), reducing the density of pest flies and improving soil pasture. No non-target effect of dung beetles has ever been recorded in Australia following these introductions. Dung beetles only feed on dung and the species selected have strong preference for cattle and sheep dung. Due to their strict diet, no risks to human, native fauna and flora are expected. As described in section 3.12, *E. crocatus*, *O. andalusicus*, and *G. sturmi* have a different diet to most native dung beetle fauna and therefore will unlikely compete for resources.

The most recently imported species are *Bubas bubalus* and *Onthophagus vacca*. CSIRO successfully applied to have these two species added to the Live Import List, and obtained an importation permit from DAWR for both species. *Bubas bubalus* and *O. vacca* have been released following their importation but are yet to have officially established. Import risk has been mitigated by keeping imported adult beetles in an Approved Arrangements (quarantine) facility and sterilising their eggs in Virkon® before removing them from quarantine.

Biosecurity Australia has not undertaken any Import Risk Analyses specifically for *E. crocatus*, *O. andalusicus*, or *G. sturmi*. A permit issued by the Department of Agriculture and Water Resources (DAWR) will be required to import these species as they are classed as ‘biological control agents with targets other than plants’. CSIRO currently holds an importation permit for *O. vacca* as further importation of this species is part of the Rural Research and Development for Profit program ‘Dung Beetles as Ecosystem Engineers’.

Euonthophagus crocatus, *O. andalusicus*, and *G. sturmi* were assessed by CSIRO as part of an initial research program to find a suitable late-winter to early-spring active dung beetle for introduction to Australia. These species were recommended above other winter and spring-active dung beetles for varying reasons. *Euonthophagus crocatus* was recommended due to its abundance at various sites in Morocco and its tolerance of a range of seasonal conditions. *Onthophagus andalusicus* was recommended due to its abundance various sites in Morocco and the ease of breeding observed during a pilot breeding project at CSIRO Montpellier. *Gymnopleurus sturmi* was recommended due to its abundance in Morocco and Tunisia and its ability to disrupt dung more strongly than other roller species by feeding at the dung pat (Professor Jean-Pierre Lumaret pers. comm.).

7. Assess the likelihood that the species could establish a breeding population in Australia

The aim of importing *E. crocatus*, *O. andalusicus* and *G. sturmi* in Australia is for them to establish breeding populations and fill the seasonal and geographical gaps that exist in introduced dung beetles to control dung.

7.1: Ability to find food sources

Dung beetles use odour to locate dung. Most dung beetle species forage close to the ground, finding dung either by actively flying around and searching or by sitting and waiting for ‘odour trails’ to reach them. Once located the dung pat is approached by flight (Cambefort and Hanski 1991). As *E. crocatus*, *O. andalusicus* and *G. sturmi* will be introduced directly to livestock pastures they should not have any issue finding food.

Exotic dung beetles introduced to Australia seem to disperse depending on where livestock dung is found. For example, introduced *Bubas bison* were found to remain in their original cattle pasture in areas of permanent stocking, only moving between pads. In a different area, a colony of the same species was found to leave the original paddock to follow cattle being moved to paddocks up to a kilometre away (Doubé and Marshall 2014). As for other dung beetle species, *E. crocatus*, *O. andalusicus* and *G. sturmi* are expected to disperse from their original site to follow their food source.

7.2: Ability to disperse over long distances

The exact rate of dispersal for *E. crocatus*, *O. andalusicus* and *G. sturmi* is unknown. The ability of these species to disperse through southern Australian will be unclear until after their introduction and establishment. Dung beetles previously introduced to Australia by CSIRO show varying capability for dispersing over long distances (Ridsdill-Smith and Edwards 2011). During previous releases CSIRO has re-distributed dung beetle species in order to encourage dispersal in species that did not naturally reach the limits of their expected

distribution (Edwards 2007). The same practice will be used if necessary in the introduction of *E. crocatus*, *O. andalusicus* and *G. sturmi*.

7.3: Ability to survive and adapt to different climatic conditions

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* were selected for introduction based on their environmental requirements matching with climatic conditions in southern Australia, particularly in the south-west. Environmental niche modelling was performed for the three species, comparing the climate of their native range (Section 3.4) with Australian conditions (figures 13, 14, 15).

The program DIVA-GIS was used with bioclimatic variables to run the ‘ecological niche modelling’ function. A non-conservative model of four bioclimatic variables (mean temperature of the warmest quarter, mean temperature of the coldest quarter, precipitation of warmest quarter, precipitation of coldest quarter) was used, rather than the full set of 19, so the modelled habitats are broad. Non-conservative models were used in environmental niche modelling for foreign dung beetle species previously introduced by CSIRO (Edwards 2007).

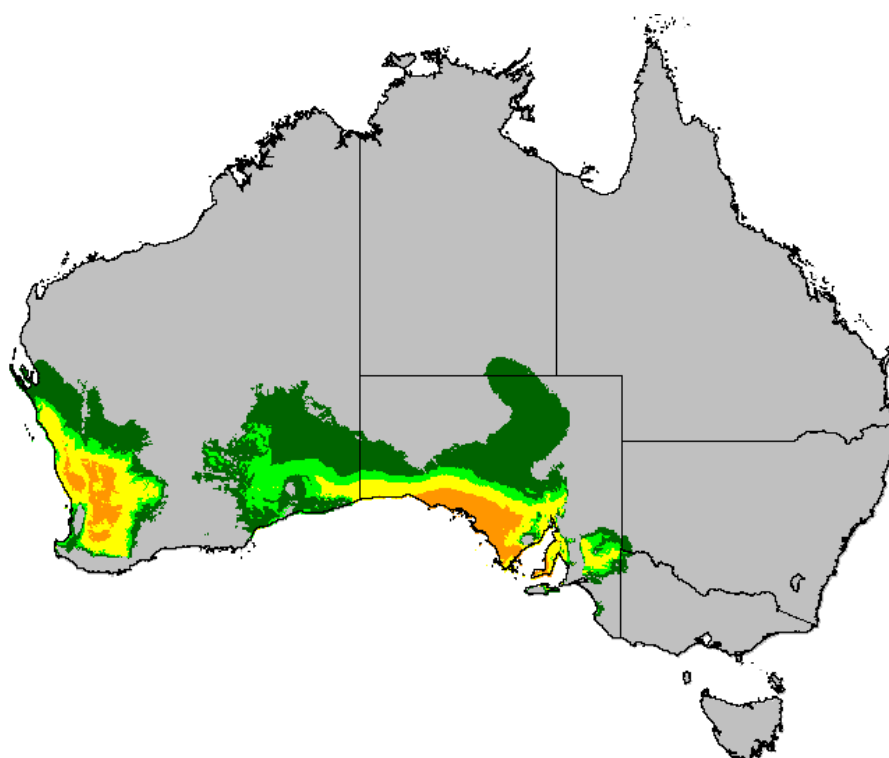


Figure 13. Potential establishment area of *Euonthophagus crocatus* in southern Australia (Favourability in decreasing order: red, orange, yellow, lime green, dark green).

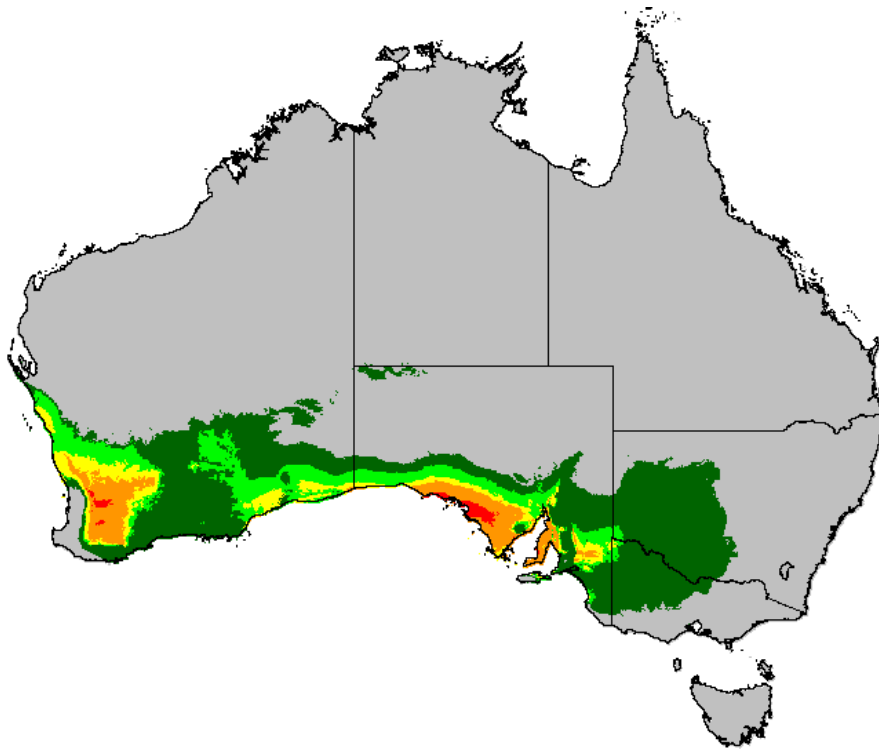


Figure 14. Potential establishment area of *Onthophagus andalusicus* in southern Australia (Favourability in decreasing order: red, orange, yellow, lime green, dark green).

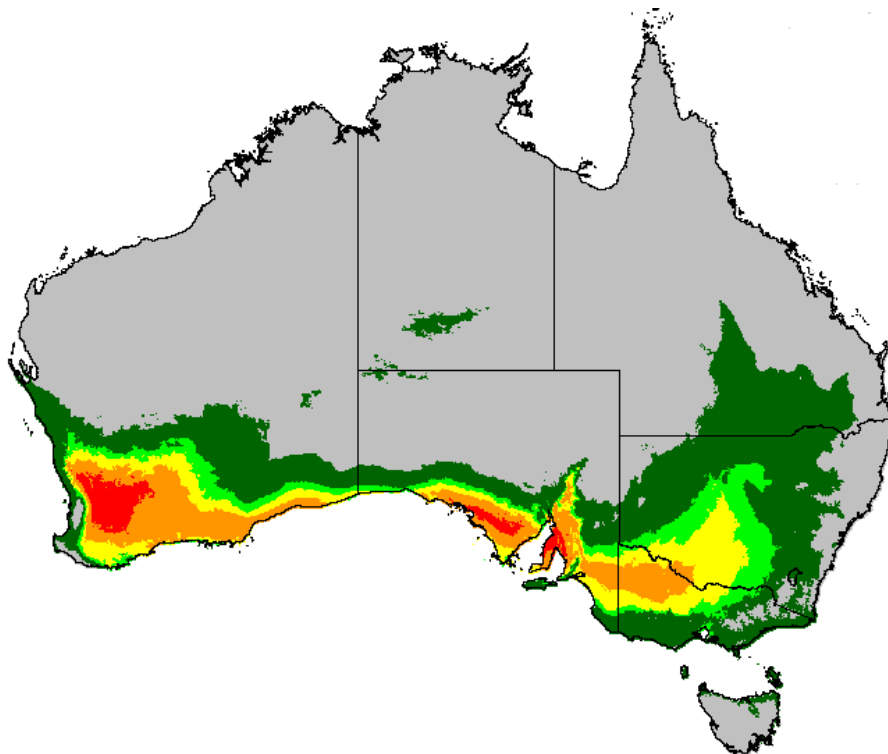


Figure 15. Potential establishment area of *Gymnopleurus sturmi* in southern Australia (Favourability in decreasing order: red, orange, yellow, lime green, dark green).

If *E. crocatus*, *G. sturmi* and *O. andalusicus* are capable of dispersing over a long distance they are not likely to thrive outside the modelled area due to the climate being unsuitable (Doubé *et al.* 1991). This is supported by data from previous foreign dung beetle releases. Observations of established species are usually found within the area outlined by the niche model, typically within the less restrictive (4-5 variables) model (Edwards 2007).

All three species are not very active across the colder seasons (section 3.13) and may go into diapause to avoid cold conditions (section 3.6). Many species of Mediterranean dung beetles engage in diapause to avoid the very dry summer (Lumaret and Kirk 1991), but *E. crocatus* and *G. sturmi* are active during early to mid-summer which indicates some tolerance for dry conditions. *Euonthophagus crocatus* is not found in Saharan regions (section 3.4) so there must be a limit to its tolerance. *Gymnopleurus* species seem to be relatively tolerant of drought (Cambefort 1991a), and *G. sturmi* shows a preference for dry sites (section 3.7). However, *G. sturmi* is also not distributed in desert regions (section 3.4) so it must need some rainfall. *Onthophagus andalusicus* has a shorter summer activity period than the other species (early summer) and favours wetter areas (section 3.7) so may not be as drought-tolerant. On the other hand, *E. crocatus* and particularly *G. sturmi* could probably tolerate periods of drought but may not survive extended dry periods.

7.4: Ability to find shelter

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are dependent on dung to construct shelters for their young. Wherever livestock dung and open areas are available these dung beetle species will be able to find shelter. The process of constructing nests for reproduction is described in section 4.2. Developing dung beetles benefit from the protection provided by their brood ball within the nest (section 7.5). Mature dung beetles presumably also benefit from the shelter provided by deep nesting tunnels. *Euonthophagus crocatus* and *O. andalusicus* construct feeding tunnels during the maturation feeding period, which is described in section 4.1. These shallow tunnels do provide some shelter, but immature adults can be vulnerable to predation by animals such as foxes and ibis (Doubé and Marshall 2014).

Onthophagus andalusicus is not likely to be found in cities and towns due to a lack of its preferred food source and habitat. *Onthophagus andalusicus* was found to be very rare in urban Rome, which Fattorini (2011) attributed to a decline in grazing. *Euonthophagus crocatus* and *G. sturmi* could occupy urban habitats due to their consumption of dog faeces. However, their reproduction would be inhibited as dung beetles use herbivore dung for breeding (Cambefort and Hanski 1991) (section 3.8). Dung beetle activity is likely also inhibited by the poor quality and small amount of dog faeces, the distance between faeces piles, and the use of veterinary chemicals in dogs (Northern Tablelands Dung Beetle Express 2009).

7.5: Rate of reproducing

As stated in section 4, information on the specific reproductive behaviour of *E. crocatus*, *O. andalusicus* and *G. sturmi* is scarce, so the rate of reproduction of these species must be assumed based on information about their breeding pattern, family, or genus. The current studies on these species in the CSIRO European Laboratory will determine their likely rate of reproduction prior to their release into the Australian environment.

The rate of reproduction of the three species is uncertain. They nest multiple times during their active season, as is the case with species in their genus or breeding pattern (section 4.2 and 4.3). The number of eggs laid by *E. crocatus* and *O. andalusicus* is unknown, and varies within the Onthophagini tribe. Under laboratory conditions, *O. vacca* was shown to produce 10-20 brood balls during their lifetime (Wright et al. 2015). Similar numbers would be expected for *E. crocatus* and *O. andalusicus* which have similar sizes and life strategies. *Gymnopleurus sturmi* should lay 1-2 eggs per nest like other species within its genus (section 4.6), but the total number of nests produced in a year is unknown.

Like other dung beetle species, the reproduction rate of these species will be limited to their active season. The seasonal quality of dung is observed to affect the fecundity of species introduced to Australia, with spring dung encouraging the greatest productivity (section 4.2). This will benefit *E. crocatus*, *O. andalusicus* and *G. sturmi* as they typically breed during spring. Temperature and rainfall are presumably a factor in triggering the active season for these species, but the extent of this is unclear (section 4.2).

Reproductive competition from other dung beetle species is not expected to be an issue given the lack of winter and spring-active introduced dung beetles in the expected Australian distribution of *E. crocatus*, *O. andalusicus* and *G. sturmi* (section 10.2), and the different niche occupied by native dung beetle species (section 3.12).

Population increase in dung beetles is also dependent on the survival of offspring. Dung beetle offspring benefit from being protected by their brood ball or brood mass within the nest, and so are free from competition, the need to find food, or typical variations in temperature (Halffter and Edmonds 1982). However, species that do not care for their brood balls leave them vulnerable to invasion by parasites and disease (section 3.11). The offspring of *E. crocatus*, *O. andalusicus* and *G. sturmi* should benefit from the protection of their brood chamber, but will be vulnerable to parasites and disease due to a lack of parental care.

7.6: Increased potential for population establishment due to a greater number of individuals

Introduced dung beetles seem to be less likely to establish in Australia when release numbers are lower. Of the 20 foreign species released by CSIRO that failed to establish, 18 of 20 of these had fewer than 8000 individuals released. In contrast, for 20 of the 23 species that did

establish more than 8000 individuals were released. The reason for the release of low numbers of some species was generally due to only a small number of eggs being imported from overseas (for species bred from eggs) or due to difficulties in breeding in quarantine. These issues may reflect the life history strategy of species with a low reproductive rate and developmental delays (Edwards 2007). All efforts will be made to breed as many individuals as possible for release in order to maximise the chances of establishment.

7.7: Characteristics that the species has which could increase its chance of survival in the Australian environment

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* will benefit from the availability of their preferred habitat and climate conditions (section 7.3 figures 13, 14, 15). They will also benefit from the abundance of their preferred dung types, as Australia has many sheep and cattle distributed through southern Australian pasture (section 3.8). *Euonthophagus crocatus*, *O. andalusicus* and *G. sturmi* will also benefit from a lack of competition from native dung beetle species (section 3.12). Furthermore, these three species will be specifically introduced to fill seasonal and geographical gaps. The activity periods of introduced dung beetle species that are already established in southern Australia do not completely overlap with the activity periods of *E. crocatus*, *O. andalusicus* and *G. sturmi* (section 10.2 table 1), so the three species should not be suppressed due to resource competition. Their expected diapause period of *E. crocatus*, *O. andalusicus* and *G. sturmi* may allow individuals to survive harsh seasonal conditions, although the specifics of this period are not known (section 3.6).

7.8: Limiting factors that may constrain Australian populations of the species

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* were selected based on their ability to disperse dung and climate matching suitability. Although these species will be introduced to their preferred climate type (section 7.3), they are not expected to thrive outside the modelled area due to the climate being unsuitable. Once introduced, they will have to adapt to the conditions of their new environment to allow establishment. Other factors that may limit establishment include poor timing of introduction, low number introduced, the use of parasiticides on cattle and sheep, environmental conditions such as drought, presence of predators, diseases and parasites, and poor dung quality present. Predation has been observed in previously established colonies of introduced dung beetles but is not considered to be a limiting factor due to persistence of colonies even following heavy predation (section 3.11).

8. Provide a comprehensive assessment of the potential impact of the species should it become established in Australia

8.1: Similar niche species

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not likely to compete with native dung beetle species due to their different niche preferences. *Euonthophagus crocatus*, *O. andalusicus*, and *G. sturmi* generally utilise sheep and cattle dung in open habitats, while native dung beetles are adapted to marsupial dung in forest or woodland habitats (section 3.12). Some native dung beetle species of southern Australia (e.g. *O. ferox*) are occasionally found in livestock dung, but there is a lack of conclusive evidence that these species are excluded through competition with introduced species (section 3.12). These species are expected to persist due to their ability to continue to utilise marsupial dung in forest or woodland habitats, which is the dung they evolved with (section 3.12).

Several foreign dung beetle species have already been introduced to southern Australia and have similar food and habitat requirements to *E. crocatus*, *O. andalusicus* and *G. sturmi*. However, the activity period of these pre-existing species does not completely overlap with the activity period of the three new species (section 3.13, section 10.2 table 1), hence why they are being considered for introduction, so *E. crocatus*, *O. andalusicus* and *G. sturmi* should not be prevented from accessing resources through competition.

8.2: Susceptibility to or ability to transmit any pests or diseases

The importation process for adult *E. crocatus*, *O. andalusicus*, and *G. sturmi* will mitigate the risk of pest or disease transmission by starving and cleaning beetles collected from northern Africa and south-western Europe prior to shipping, and by keeping the imported adults in an Approved Arrangements (quarantine) facility for the duration of the project. Only eggs sterilised in Virkon® will be allowed out of the quarantine facility (see sections 9 and 11).

Dung beetles have been found to destroy pathogens in dung through altering the conditions within dung pats, through digestion, and by burying dung in the soil (Doubé and Marshall 2014; Nichols *et al.* 2017). Pathogens destroyed by dung beetles include *Cryptosporidium parvum* and *Escherichia coli*, both of which pose a risk to humans (Mathison and Ditrich 1999; Ryan *et al.* 2011; Jones *et al.* 2015), and livestock intestinal worms such as those in the *Trichostrongylus* family (Doubé and Marshall 2014). In future, introduced dung beetles could be used as part of an integrated approach to control intestinal parasites. This would be helpful to Australian farmers who host dung beetle colonies on their property, as they are not able to use most cattle parasiticides (e.g. drenches) due to their potential to poison dung beetle adults and larvae (Doubé and Marshall 2014).

However, dung beetles may be able to disseminate pathogens that survive their digestive tract or attach to their bodies (Nichols *et al.* 2017). Individual dung beetles that are carrying pathogens should not typically come into contact with people due to their preferred food source and habitat. In case of contact humans should take common-sense precautions such as not handling dung, dung beetles, or pasture soil. If handling is necessary people should wash their hands with soap and water afterwards (Centers for Disease Control and Prevention 2009).

Xu *et al.* (2003) has suggested that ball-rolling may spread pathogens contained in the dung ball, contaminating the soil. This should not be a major concern because as roller beetles do not bury their dung ball far from the original dung pat, they are not likely to spread pathogens beyond the pasture they picked them up in. The precautions referred to in the previous paragraph also apply here.

8.3: Probable prey/food sources, including agricultural crops

Euonthophagus crocatus, exclusively feed on dung, so will not prey on animals or plants.

8.4: Habitat and local environmental conditions

Euonthophagus crocatus, are not likely to cause soil erosion through a decrease in vegetation cover. They do not feed on vegetation, and their small body size (*E. crocatus* 6-12mm long, *O. andalusicus* 6-12mm long, *G. sturmi* 10-15 mm long) and downward-tunnelling should not disrupt vegetation cover. In fact, they have been shown to improve soil conditions and encourage pasture growth through dung burial behaviours. More information on these effects is given in sections 10.3 and 8.7. As *E. crocatus*, *O. andalusicus* and *G. sturmi* have not been introduced to Australia previously they have not been recorded causing damage to any native environments or to forestry and agriculture. They are not considered likely to do so due to their preference for livestock dung in pastoral habitats, and in fact appear to have a positive effect on primary production (section 8.7). No reports of these species causing damage in their native range were found in literature.

Dung beetle species are known to disperse seeds through the transport and burial of the dung of primary dispersers (Estrada and Coates-Estrade 1991, Shepherd and Chapman 1998, Andresen and Levey 2004, Koike et al. 2012 cited in Griffiths *et al.* 2016). Seeds can survive digestion by sheep and cattle although their viability may be reduced (Hogan and Phillips 2011), so it is possible that *E. crocatus*, *O. andalusicus* and *G. sturmi* could spread weeds through dung dispersal within the original pasture. However, dung beetle seed dispersal has been shown to have varied effects on germination success. Based on experimentation it seems that shallow dung burial assists germination, deep burial inhibits germination, and surface dispersal may have either effect (Estrada and Coates-Estrade 1991, Shepherd and Chapman 1998, Koike et al. 2012 cited in Griffiths *et al.* 2016) (D'hondt *et al.* 2008).

Gymnopleurus sturmi seems most likely to disperse seeds as it rolls dung balls and nests just below the soil surface (section 4.2). Roller species are believed to reduce space and resource competition for seedlings through their dispersal behaviours (D'hondt *et al.* 2008).

Euonthophagus crocatus and *O. andalusicus* will probably not disperse seeds well, as the deep tunnels used for nesting will inhibit germination. The use of shallow maturation feeding tunnels should assist germination, but if the host dung pat remains on the soil surface this may prevent the seedling from surviving.

It is also possible that *E. crocatus*, *O. andalusicus* and *G. sturmi* could disperse small weed seeds attached their body. The dispersal range of these species is unknown but could potentially be quite wide (section 7.2). No research on seed dispersal by dung beetles through this mechanism was found in literature. It should be noted that weed dispersal by introduced dung beetles has not been reported in existing dung beetle project literature. If introduced dung beetles were highly effective dispersers of weeds property owners likely would have made CSIRO aware of this during the past 50 years of the project.

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not likely to inhibit tree seedling germination success through dung dispersal in native forests and woodlands because they do not inhabit this niche, preferring livestock dung in open habitats.

8.5: Any control/eradication programs that could be applied in Australia if the species was released or escaped

As *E. crocatus*, *O. andalusicus* and *G. sturmi* are intended to be released as a biological control measure for bush flies and dung and are not considered likely to become an environmental pest, no control/eradication programs are considered necessary outside of the existing import risk precautions taken by CSIRO (see sections 9 and 11).

8.6: Characteristics or behaviour of the species which may cause land degradation

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not likely to degrade soil through their dung burial behaviours due to their small body size. Instead, these species are expected to aerate and mix the soil, increase water permeability, and improve soil nutrient levels through tunnelling and dung burial behaviours. Evidence for these positive benefits has been found in and outside of Australia (see section 10.3).

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not likely to have a negative effect on wetlands. These species eat dung exclusively and will not eat wetland vegetation. *Onthophagus andalusicus* does favour wetter sites (Verdú *et al.* 2015) and so may engage in dung burial behaviour near wetlands if suitable habitat and food are available. As stated in section 8.4, tunnelling behaviour by this species will disturb the substrate but studies on the effects of dung beetle activity in the soil of southern Australia have so far not shown negative effects, but rather an improvement in soil quality and pasture production.

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not likely to pollute water bodies through their use of dung. Any dung removed from the pad is buried in situ (*E. crocatus* and *O. andalusicus*) or a short distance away (*G. sturmi*). Dung beetle activity is hoped to reduce the risk of water pollution by preventing nutrient runoff from dung left on the soil surface (Edwards 2007). This has been demonstrated in simulated rainfall experiments in southern Australia by Doube (2008).

8.7: Impacts on primary industries

There has been no reports of *E. crocatus*, *O. andalusicus* and *G. sturmi* causing damage to livestock, poultry, or agriculture. They eat dung and nest in the soil (sections 3.8, 3.7, 4.2) so will not eat or damage crops, garden plants, trees, or stored grain.

Dung beetle introduction to southern Australia is hoped to benefit pasture productivity through incorporating dung nutrients into the soil, aerating and mixing the soil, and increasing water permeability. An increase in productivity has been observed by Doube in his experimentation on the effects of dung beetles on soil in southern Australia. For example, pasture production was 30% greater in plots with dung beetle activity than plots without over a 9 year study at Kuitpo, SA (Doube and Marshall 2014). Increases in plant growth have also been observed in laboratory conditions (Bornemissza and Williams 1970, Fincher et al. 1981, Yokoyama et al. 1991, Bertone et al. 2006, Nichols et al. 2008 cited in Doube 2018). CSIRO is currently participating in a project under the Rural Research and Development for Profit program to gain a more thorough understanding of the ways that dung beetles can assist primary industry (Department of Agriculture and Water Resources 2018b).

As described in section 8.2, the ability of dung beetles to destroy intestinal worms such as those in the *Trichostrongylus* family could assist farmers as part of an integrated approach to controlling livestock parasites.

8.8: Damage to property

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not likely to damage property. As pasture-dwelling species with a definite preference for livestock dung they will not have any need to e.g. inhabit buildings.

8.9: Is the species a social nuisance or danger?

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not likely to be a social nuisance or pose a danger to the public as they inhabit a niche usually only accessed by livestock and property owners.

8.10: Any potential threat to humans.

Dung beetles cannot bite humans (section 3.12) and appropriate quarantine measures will be taken to prevent disease or pest transmission by imported beetles (sections 9 and 11). Dung

beetles have been found to destroy pathogens that are harmful to humans including *E. coli*, but pathogens may survive digestion or be carried on the beetle's body. People who need to enter pastures should try to refrain from handling dung beetles, dung, or pasture soil, and wash their hands thoroughly if they need to do so (section 8.2).

9. Conditions or restrictions applied to the import of the species to reduce any potential negative environmental impacts

Potential negative impacts from the importation of *E. crocatus*, *O. andalusicus* and *G. sturmi* can be reduced by following correct importation practises and quarantine procedures.

Adult beetles of each species will be collected from various localities and air freighted to Australia (up to 500 male-female pairs per consignment). Relevant to the status of *G. sturmi* as Near Threatened in Europe, adults of this species will only be collected from localities in northern Africa where the species is very abundant. Likewise, *E. crocatus* and *O. andalusicus* will only be collected from localities in which they are abundant, i.e. northern Africa for *E. crocatus* and northern Africa and south-western Europe for *O. andalusicus* (section 3.4). Following collection from the wild, adult beetles will be starved for three days to prevent contamination from foreign parasites they may have eaten. They will be washed in plain water and any parasites on their bodies will be picked off. The adult beetles will be loosely packed in containers of loose vermiculite, and shipments of the beetles will be sent using an accredited live animal courier. After arrival the imported adult beetles will be kept in an Approved Arrangements (quarantine) facility for the duration of the project. Any dung beetles that die in quarantine will be autoclaved before disposal, and all eggs that are taken from quarantine to be raised will first be sterilised in Virkon®.

These importation and quarantine restrictions will follow DAWR conditions and reduce the risk of negative environmental impacts from parasites or the accidental release of imported beetles. Additional information on collection, importation, and quarantine practises can be found in section 6 and section 11.

10. Summary of proposed activity

10.1: Aims and history of the CSIRO dung beetle project

The objective of introducing *E. crocatus*, *O. andalusicus* and *G. sturmi* is to enhance dung burial and bush fly (*Musca vetustissima*) control in the cattle and sheep farming areas of southern Australia, with a focus on the south-west. Although many of the 23 species of exotic dung beetle already established in Australia are regarded as being spring-active (section 10.2 table 1), few emerge early enough to have a significant influence on the rapid increase in bush fly populations seen in southern Australia that occurs from August-October (Hughes 1970). Following an initial research project and using environmental niche modelling CSIRO has identified *E. crocatus*, *O. andalusicus* and *G. sturmi* as non-native dung beetle species

with the potential to fill these gaps in beetle activity. All three species are active from August-October and are appropriate for the climate in southern Australia (section 3.13, section 7.3 figures 13, 14, 15). Introduced dung beetles suppress fly activity by disturbing the dung pats flies use to breed. They also benefit the environment by removing dung from the soil surface. Australian native dung beetles do not control livestock dung and dung breeding flies effectively (Doubé *et al.* 1991), as most species are adapted to the use of marsupial dung in forest or woodland habitats (section 3.12).

The use of exotic dung beetles for the biological control of bush flies was first proposed in the early 1960s by CSIRO Entomology. The project was based on the importation of adult beetles and surface-sterilised eggs from Hawaii, Africa, and Europe and ran until 1986, resulting in the field release of over 40 species of scarabaeine dung beetle, 23 of which are now regarded as being established. A second round of introductions, which relied on the importation of adult beetles from Europe, was undertaken from 1990-1992 (Edwards 2007). A third round of introductions also relying on adult imports from Europe was undertaken in 2011-2015 (Wright *et al.* 2015). The current round of the project has run from 2018 and has relied on the importation of adult beetles (*O. vacca*) from Europe and northern Africa.

Previous dung beetle introductions have focused exclusively on cattle dung burial (Edwards 2007). However, unburied sheep dung also fouls pastures (Skrijka 1987) and bush flies will breed in sheep dung (Ferrar 1979). *Euonthophagus crocatus*, *O. andalusicus*, and *G. sturmi* all utilise sheep dung, so will likely provide control for sheep farming areas as well as cattle farming areas. This will be of benefit to southern Australia given the concentration of sheep farming across the region (section 3.8, figure 12).

Information on the specifics of the dung beetle breeding program (e.g. segregating males) is given in section 11.

10.2: Established exotic dung beetles

An analysis of dung beetle records in Australia has shown that most pastoral areas of Australia are now home to one or more species of introduced dung beetle. In tropical and sub-tropical pastures, the number of introduced species is commonly as high as 6-10, but in southern Australia, there are usually fewer species, particularly those that are active in late winter and early spring (Edwards 2007). This supports the observations of pastoralists and Landcare groups across Australia, particularly in the southern states, that there is an important seasonal gap in dung beetle activity in late winter to early spring.

Only four (*Bubas bison*, *Copris hispanus*, *Onitis caffer*, *Onthophagus binodis*) of the 15 established introduced species in southern Australia are found in the south west (table 1). *Bubas bison* activity in the south west declines sharply after mid-winter, so this species is not providing sufficient bush fly control for this region. *Copris hispanus* is active in spring, but has only been observed at its original release site. *Onitis caffer* is active in winter and spring

but is not widely distributed. *Onthophagus binodis* is well distributed, but does not become active until mid to late-spring.

Only two (*Bubas bison*, *Onitis caffer*) of the 13 established introduced species in south eastern Australia are active at all in the August/September period (table 1), and since both species are relatively recent introductions from Western Australia into southeast Australia, neither is thought to be providing any substantial level of control of dung at this time. As noted, *Bubas bison* and *Onitis caffer* are more abundant in Western Australia where the activity of *Bubas bison* declines sharply after mid-winter.

Table 1. Status and activity period of introduced dung beetles in southern Australia. [Data from (Edwards 2007; Dung Beetles for Landcare Farming Committee 2008; John Feehan and Belinda Pearce pers. comm. cited in Wright and Wardhaugh 2009; Edwards *et al.* 2015)].

| Species | Activity period | Status in southern Australia |
|----------------------------------|---------------------|---|
| <i>Bubas bison</i> | Apr/May to Aug/Sept | Localised but spreading fast. Breeds in spring in south east Australia but finishes by mid-winter in south west WA. |
| <i>Copris hispanus</i> | Mar to Nov | Found at original release site in WA. |
| <i>Euoniticellus africanus</i> | Sept/Oct to Mar/Apr | Localised in south east. |
| <i>Euoniticellus fulvus</i> | Oct/Nov to Feb/Mar | Common in south east, localised in south west. |
| <i>Euoniticellus intermedius</i> | Oct/Nov to Mar/Apr | Common in south east but absent in southern coastal regions. Localised in south west. |
| <i>Euoniticellus pallipes</i> | Oct to Apr | Well distributed in south east and south west. |
| <i>Geotrupes spiniger</i> | Dec to May/June | Localised in south east but spreading fast. |
| <i>Onitis alexis</i> | Dec to Mar | Well distributed in south east, localised in south west. |
| <i>Onitis aygulus</i> | Nov to Feb | Localised but spreading fast. |
| <i>Onitis caffer</i> | Apr/May to Aug/Sept | Rare in south east, but locally abundant in south west. |
| <i>Onitis pecuarius</i> | Nov/Dec to Apr/May | Localised in south east. |
| <i>Onthophagus binodis</i> | Oct/Nov to Feb/Mar | Common in south east and south west. |

| | | |
|---------------------------------|----------------------------------|--|
| <i>Onthophagus gazella</i> | Oct to Apr | Well distributed in south east, absent in south west. Not suitable for colder areas. |
| <i>Onthophagus nigriventris</i> | Nov to Mar (dung beetle ID book) | Localised in south east. |
| <i>Onthophagus taurus</i> | Oct to Nov | Common in south east and south west. |

10.3: Benefits of dung burial

The introduction of *E. crocatus*, *O. andalusicus* and *G. sturmi* is intended to reduce pasture fouling through dung burial. Unburied dung is a source of annoyance to farmers. Cattle dung may remain on the soil surface for 6-12 months (Weeda 1967). Waterhouse (1974) estimated that the annual loss of productive pasture from unburied dung voided by a single cow to be about 0.1 hectare. With the national herd running at about 26 million (Australian Bureau of Statistics 2018), the overall loss of pasture approximates to 2.6 million hectares per year. In a Polish study Skrijka found that unburied sheep dung will cover around 20% of a pasture in a year (1987). In areas of heavy stocking without dung beetle activity, farmers sometimes have to harrow the paddocks to break up and disperse the pads.

Dung of similar characteristics to cattle dung is also produced by zebu cattle (*Bos indicus*) and domestic hybrids between *Bos taurus* and *Bos indicus*. *Ovis aries* is the only widely distributed sheep species found in Australia (Atlas of Living Australia 2015). Dung of similar characteristics to sheep dung is produced by goats (*Capra hircus*). Marsupial dung is dropped as pellets of varying size but is much smaller and drier than the typical cattle dung pad or sheep pellets.

One of the expected secondary benefits of the dung beetle project was dung burial improving soil and the surrounding land. Expected improvements included improvement in soil structure and nutrient levels and a reduction of nutrient runoff into waterways. Experimentation by Doube in southern Australia found that introduced *B. bison* beetles did benefit the soil and the surrounding landscape through incorporating nutrients, aerating and mixing the soil, increasing water permeability, and preventing nutrient runoff from unburied dung (Doube 2008; Doube and Marshall 2014). Improvements in nutrient levels and soil structure through dung beetle activity have been observed outside Australia (Nichols *et al.* 2008; Brown *et al.* 2010). Field and lab studies have also found an increase in plant productivity in soil occupied by dung beetles (Doube and Marshall 2014; (Bornemissza and Williams 1970, Fincher *et al.* 1981, Yokoyama *et al.* 1991, Bertone *et al.* 2006, Nichols *et al.* 2008 cited in Doube 2018), which suggests that dung beetles may assist crop production (section 8.7). CSIRO is currently participating in a project under the Rural Research and Development for Profit program to gain a more thorough understanding of the ways that dung beetles benefit the soil and improve crop productivity (Department of Agriculture and Water Resources 2018b).

10.4: Control of *Musca vetustissima*

Musca vetustissima is endemic to Australia. Bush flies are present throughout the year in the drier parts of tropical and sub-tropical Australia (Hughes 1970), but are unable to survive over winter in southern parts of the continent. These areas are re-colonised during late-winter and spring by migrants from the north. The arrival of bush flies in southern Australia in August-October commonly precedes the emergence of already established spring-active exotic beetles by 1-2 months (section 10.2 table 1). Dung pollution is common at this time of the year and so fly populations build up rapidly, hence the need for introducing winter and spring-active beetle species.

Following their migration from northern Australia bush flies take advantage of the nutritious dung of cattle and sheep (section 4.2). Bush flies lay their eggs in cracks in the base of dung pads, and their hatched larvae feed on the dung juices. This leads to a rapid increase in bush fly populations during early spring. Dung beetles suppress this activity through their own use of dung for nesting and feeding. Dung beetles stir up the dung pad as they collect dung for nesting, smothering bush fly eggs. The process of removing dung for nesting and feeding on the dung juices dries out the pad, starving the bush fly larvae (Edwards *et al.* 2015).

Bush flies have been implicated in the transmission of trachoma in Aboriginal communities in north-western Australia (Da Cruz *et al.* 2002). The species is more widely known as a major nuisance pest of humans and livestock, but no formal assessments have been made of its economic impact. Nevertheless, the existence of the current outdoor dining culture has been credited to the activity of dung beetles in suppressing bush fly populations throughout Australia for much of the year (Doubé and Marshall 2014).

The introduction of exotic dung beetles for the biological control of dung and dung breeding flies is widely regarded among Australian livestock producers as being highly successful. For example, dairy farmers have benefitted from the absence of flies from the milking shed during summer (Doubé and Marshall 2014). However, control of flies has been difficult to document scientifically. Laboratory studies regularly demonstrate effectiveness of dung beetles to reduce fly breeding (e.g. Hughes *et al.* 1978). It has been much more difficult to demonstrate under natural situations with entire dung beetle assemblages (e.g. Feehan *et al.* 1985; Tyndale-Biscoe and Vogt 1996) due to the complications of fly migration, seasonal weather, parasiticide usage and dung quality, as well as the changing seasonal abundance of dung beetle species and predators. Some authors have been able to demonstrate real reduction in flies emerging from dung pads due to the activity of dung beetles (Ridsdill-Smith and Matthiessen 1984, 1988; Fay *et al.* 1990) or inferred this from the altered age structure of flies that indicated reduction of local fly breeding (Tyndale-Biscoe and Vogt 1996).

10.5: Control of other dung-breeding muscoid flies

Although control of other dung-breeding muscoid flies is not a goal of the dung beetle project, there are two other fly species that could potentially be affected by the introduction of *E. crocatus*, *O. andalusicus* and *G. sturmi*

Of the 26 species of muscoid flies (Table 2) discussed in the major work on larvae that breed in cattle dung in Australia (Ferrar 1979), 13 are non-endemic, including the pests *Musca domestica* (house fly), *Haematobia irritans exigua* (buffalo fly) and *Stomoxys calcitrans* (stable fly). The endemic fly species evolved before the introduction of domestic cattle and must therefore have utilised the dung of native marsupials and have adopted bovine dung as a secondary resource. Of the 13 endemic species, 10 occur in southern Australia, the region being targeted in this program. Only 3 of these 10 southern species are primarily found in open pasture or open woodland habitats, the rest being more specialised in woodland or forest habitats.

Table 2. Information on dung-breeding muscoid flies in Australia. Data mostly from Ferrar (1979) with nomenclature updated using Evenhuis (1989).

| Scientific and [common] name | Geographical distribution | Habitat | Adult food, biological notes | Endemic | Southern Australia | Pasture (P) or forest (F) |
|---|--|---|--|---------|--------------------|---------------------------|
| <i>Brontaea (Gymnodia) obliterata</i> (Malloch) | Basically southern but extending just into the tropics in coastal areas | Open pasture and open woodland | Primarily associated with emu dung | Y | Y | P |
| <i>Musca vetustissima</i> Walker [bush fly] | Throughout Australia, including Tasmania, but absent southern regions in winter; also PNG | Open pasture, open woodlands | Sweat and body secretions of humans and livestock, dung | Y | Y | P |
| <i>Neomyia (Orthellia) australis</i> (Macquart) | Commonly in eastern Qld and NSW; less common in NT and PNG (more temperate distribution than <i>N. lauta</i>) | Open pasture and woodland, sometimes denser forests | Collected from vegetation, flowers, human faeces. Reared from bovine dung. Adults abundant January to May. Hovers around faecal matter in the bush and | Y | Y | P |

| Scientific and [common] name | Geographical distribution | Habitat | Adult food, biological notes | Endemic | Southern Australia | Pasture (P) or forest (F) |
|---|---|--|---|---------|--------------------|---------------------------|
| | | | sugary manna from the grey gum (<i>Eucalyptus</i> sp.) (Pont 1973) | | | |
| <i>Australofannia</i> species | NSW, Vic, Tas | Wet sclerophyll forests | Attracted to cattle dung baits | Y | Y | F |
| <i>Helina</i> species (not well understood) | Eastern Australia, temperate areas | Likely restricted to woodland habitats or forests | Attracted to cattle dung baits | Y | Y | F |
| <i>Metopomyia atropunctipes</i> Malloch | Eastern NSW, Victoria, Tas | Mountain forests | Attracted to cattle dung baits | Y | Y | F |
| <i>Prohardyia carinata</i> (Stein) | Southern part of Australia | Open to closed forests; extending to open pastures at cooler times of year | Attracted to cattle dung baits, flowers | Y | Y | F |
| <i>Prohardyia macalpinei</i> Pont | South east Qld, eastern NSW, Victoria. | Fairly rare fly from forest areas | Attracted to cattle dung baits in forests | Y | Y | F |
| <i>Pyrellia tasmaniae</i> Macquart | Most common on coast and eastern ranges of south east Australia, including Tasmania, less | Woodland and forest | Sweat of humans; cattle dung. Reared from wombat droppings | Y | Y | F |

| Scientific and [common] name | Geographical distribution | Habitat | Adult food, biological notes | Endemic | Southern Australia | Pasture (P) or forest (F) |
|---|---|------------------------------------|---|---------|--------------------|---------------------------|
| | common in Qld (at altitude) | | | | | |
| <i>Musca fergusonii</i> Johnston and Bancroft | Northern Australia (Kimberley Region of WA, north half of NT, north & coastal south Qld, north Coast of NSW to just south of Grafton. Also PNG) | Open pasture, open woodlands | Sweat and body secretions of cattle, horses & humans, dung | Y | N | P |
| <i>Musca terraereginae</i> Johnston & Bancroft | Chiefly from northern tropics of Australia | Not specified | Wounds, sweat, body secretions of livestock and man; carrion, garbage | Y | N | P |
| <i>Pyrellia attonita</i> Pont | North Queensland | Open pasture and savannah woodland | Attracted to cattle dung baits | Y | N | P |
| <i>Hennigiola setulifera</i> (Stein) | Eastern Australia, Cooktown to Sydney | Medium to dense forests | Attracted to dung of cattle or humans | Y | N | F |

| Scientific and [common] name | Geographical distribution | Habitat | Adult food, biological notes | Endemic | Southern Australia | Pasture (P) or forest (F) |
|---|--|--|---|---------|--------------------|---------------------------|
| <i>Musca domestica</i> Linnaeus [housefly] | Cosmopolitan, in association with humans | Stables, yards, other spaces with decaying/fermenting material close to human habitation | Wounds, sweat, body secretions of livestock and man | N | Y | P |
| <i>Parasarcophaga sericea</i> (Parker) | India, Sri Lanka, Burma, China, Malaysia Philippines; widely distributed in Australia | Open pastures and open woodland | Fresh cattle dung | N | Y | P |
| <i>Stomoxys calcitrans</i> (Linnaeus) [stable fly] | Cosmopolitan species; common throughout Australia associated with human settlements | Abundant around livestock yards, and stables; rarely in open pasture | Obligate blood feeders of livestock, humans, dogs and others. | N | Y | P |
| <i>Neomyia (Orthellia) timorensis</i> (Robineau-Desvoidy) | Indo-Malayan region, south through Timor, PNG etc. to Australia. In Australia, mostly east coast and eastern ranges of | Wet sclerophyll and rain-forest areas | Dung | N | Y | F |

| Scientific and [common] name | Geographical distribution | Habitat | Adult food, biological notes | Endemic | Southern Australia | Pasture (P) or forest (F) |
|--|---|---|--|---------|--------------------|---------------------------|
| | Qld to southern NSW | | | | | |
| <i>Brontaea (Gymnodia) ruficornis</i> (Malloch) | Tropical parts of Africa and Asia; in Australia, widespread through tropical north, extending s to Carnarvon in WA and Coffs Harbor in NSW | Open pasture and open woodland | Fresh cow and horse dung | N | N | P |
| <i>Haematobia irritans exigua</i> de Meijere [buffalo fly] | Indo-Australian region, from India to Japan, south to New Guinea, Australia and Fiji. In Australia, the tropical north and extending south to Brisbane. | Open pasture, but remain on animals when they enter forests | Obligate blood feeders of cattle, buffalo and horses | N | N | P |
| <i>Hydrotaea australis</i> Malloch | Tropical Australia, extending down east coast to ca | Open pasture to medium woodland | Body secretions and blood from wounds of cattle | N | N | P |

| Scientific and [common] name | Geographical distribution | Habitat | Adult food, biological notes | Endemic | Southern Australia | Pasture (P) or forest (F) |
|--|---|---|---|---------|--------------------|---------------------------|
| | Sydney. Malaya, Sri Lanka | | and horses; sweat of humans | | | |
| <i>Morellia hortensia</i> (Wiedemann) | Oriental region; in Australia in tropics with annual rainfall > 500mm | Open pasture and open woodland | Body secretions and blood (mouthparts rasp soft tissue to promote bleeding) of cattle | N | N | P |
| <i>Neomyia (Orthellia) lauta</i> (Wiedemann) | Iran east to Japan, south to Indonesia. Mainly tropical north Australia, declining in abundance to Sydney | Open pasture, open woodland in northern Australia, in dense forests in south of range | Dung | N | N | P |
| <i>Hebecnema uniseta</i> Hennig | Indonesia, PNG; in Australia from central and north Qld. | In or near rainforest or other thick forest | Attracted to cattle dung baits in forests | N | N | F |
| <i>Musca ventrosa</i> Wiedemann | Ethiopian & Oriental regions, se through Indonesia to New Guinea and | Dry to wet sclerophyll forest habitats, including swampy areas | Sweat and body secretions of humans and livestock, dung | N | N | F |

| Scientific and [common] name | Geographical distribution | Habitat | Adult food, biological notes | Endemic | Southern Australia | Pasture (P) or forest (F) |
|---|---|---------------------------------|--------------------------------|---------|--------------------|---------------------------|
| | the Solomon Islands. Tropical northern Australia, but uncommon | | | | | |
| <i>Brontaea (Gymnodia) subtilis</i> (Stein) | Africa, Indonesia; relatively uncommon in Australia: WA, NT, Qld and northern NSW | Not specified | Human and horse dung | N | N | ? |
| <i>Tricharaea brevicornis</i> (Wiedemann) | South America; in Australia from Victoria north to Grafton | Open pastures and open woodland | Attracted to fresh cattle dung | N | Y | P |

The three endemic species of southern Australia that prefer open pasture and which could be affected by the improved control of dung presented by *E. crocatus*, *O. andalusicus* and *G. sturmi* are as follows.

Musca vetustissima (Walker)

The bush fly is the secondary target for control. More information on bush flies can be found in section 10.4.

Brontaea obliterata (Malloch)

This species is mostly found in southern Australia, but its distribution does extend slightly into the tropical regions (further than the expected distribution of *E. crocatus*, *O. andalusicus* and *G. sturmi*). It is preferentially attracted to emu dung. Because of its wide distribution and dung preference it is not likely to be significantly affected by the three dung beetle species.

Neomyia australis (Macquart)

This species does breed in cattle dung in southern Australia but it is only found outside the expected distribution of *E. crocatus*, *O. andalusicus* and *G. sturmi*, in the northern coastal regions of NSW. It will not be effected by dung control provided by the three species.

It appears that additional dung burial from winter to summer in southern Australia by *E. crocatus*, *O. andalusicus* and *G. sturmi* will not have a significant undesirable effect on other muscoid flies that breed in cattle dung.

10.6: Commercial sale of introduced dung beetles

Separate from CSIRO, businesses have been set up to provide previously introduced dung beetle species such as *B. bison* to farmers looking for a way to manage dung. These businesses should not have a negative impact on the continuation of the dung beetle project. Rather, by establishing colonies of already introduced species to areas with appropriate climates and habitats this commercial activity will assist the dung beetle project aims of livestock dung burial and bush fly suppression.

The Nagoya Protocol of the Convention on Biological Diversity is likely to affect the commercial sale of introduced dung beetles by enforcing the sharing of benefits from the utilisation of genetic resources. For example, businesses that sell introduced dung beetles to farmers may be required to share profits with the countries the beetles are native to. The Nagoya Protocol should not affect the Rural Research and Development for Profit dung beetle project as the aim is for public good.

Dead specimens of *E. crocatus*, *O. andalusicus* and *G. sturmi* are sometimes sold to beetle collectors via the internet (Lumaret *et al.* 2015; Ruiz *et al.* 2015; Verdú *et al.* 2015). This is not expected to impede the establishment of these species, as such trade is minimal.

11.Guidelines on how the species should be kept

Individuals of *E. crocatus*, *O. andalusicus* and *G. sturmi* will be collected from the wild for importation to Australia. The beetles released in Australia will be captive bred at CSIRO facilities.

Adult beetles of each species will be collected from various localities and air freighted to Australia (up to 500 male-female pairs per consignment). As stated in section 9, the localities for *E. crocatus*, *O. andalusicus* and *G. sturmi* will be in northern Africa. Some collection of *O. andalusicus* may also occur in south-western Europe. It is likely that there will be a lack of genetic variation in the breeding program due to the limited number of individuals imported of each species. CSIRO will try to mitigate this issue by collecting individuals from different pasture in each collection locale, to take advantage of genetic diversity between subpopulations.

The dung beetles will be handled similarly to the previous projects in the early 1990s and 2011-2015. Following collection from the wild, adult beetles will be starved for three days to prevent contamination from foreign parasites they may have eaten. They will be washed in plain water and any parasites on their bodies will be picked off. The adults will be segregated by sex then placed in containers of moist vermiculite with breathing holes in the top. These containers will be packed loosely to enhance their chances of survival. The containers will be placed in a cooler box and sent at room temperature using an accredited live animal courier (Wright *et al.* 2015). In-coming adult beetles will be kept in an Approved Arrangement site (quarantine) in Canberra. Their eggs will be treated with a disinfecting agent (Virkon®), removed from quarantine, then either reared to adulthood and released into the wild or used for the establishment of mass-rearing colonies, the progeny of which will be released.

There are not expected to be any excess progeny in the breeding program, as all viable dung beetles will be required for release. Imported dung beetles that die in the quarantine facility will be autoclaved before being disposed of.

Males of *E. crocatus*, *O. andalusicus* and *G. sturmi* may fight for females during breeding (section 3.10). However, breeding containers in the dung beetle facilities will contain male-female pairs (1-3 pairs per box) so males are unlikely to fight due to mate competition. The breeding containers will also be provisioned with sufficient dung to reduce or eliminate ball-stealing behaviour in male *G. sturmi* (section 4.2). The containers used are 1.7 L, providing sufficient depth for the beetles to tunnel and oviposit (Wright *et al.* 2015). The number of beetles that will be kept at any time on premises is unknown and will depend on the number of beetles imported and successfully reared. Efforts will be made to prevent overcrowding, such as limiting the number of pairs kept in breeding containers as described above.

Release sites will be chosen by selecting climatically optimal sites on properties whose owners are committed to doing everything necessary to maximise the beetles' establishment, such as avoiding the use of parasiticides. Beetles will be released when they are sexually mature and physiologically synchronised with the local season. Release numbers will vary according to the numbers reared, but at any given site the aim will be to release a minimum of 500 male-female pairs of each species. Preference will be given to paddocks containing at least 50 -100 head of cattle and in which there is a well-established cattle campsite.

12. Commonwealth, state, and territory controls

Euonthophagus crocatus, *O. andalusicus* and *G. sturmi* are not prohibited by legislation or classed as a pest species by the Commonwealth (Department of the Environment and Energy 2014), NSW (search of species names and key threatening processes in <https://www.environment.nsw.gov.au/threatenedspeciesapp/>), Tasmanian (Department of Primary Industries Parks Water and Environment 2018), Qld (Department of Agriculture and Fisheries 2018), SA (Department of Primary Industries and Regions SA 2017), WA (search of species names in <https://www.agric.wa.gov.au/organisms>), Victorian (Agriculture Victoria 2018), ACT (ACT Government 2016), or NT (search of Family: Scarabaeidae in <http://pestinfo.nt.gov.au/>) governments.

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