Australian Government
Department of Climate Change, Energy, the Environment and Water

Assessment of the Impacts of 4 species of Vinegar Flies (*Drosophila* spp.) on the Environment

Consideration of an application under the Environment Protection and Biodiversity Conservation Act 1999 to amend the List of Specimens taken to be Suitable for Live Import

September 2022



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Acknowledgement of Country

We acknowledge the Traditional Custodians of Australia and their continuing connection to land and sea, waters, environment and community. We pay our respects to the Traditional Custodians of the lands we live and work on, their culture, and their Elders past and present.

Executive summary

An application was submitted to the Australian Government Department of Climate Change, Energy, the Environment and Water (DCCEEW) to amend the Live Import List to include 4 species of vinegar fly (*Drosophila* spp.) for research purposes only. The species are:

- *Drosophila azteca*
- *Drosophila bifasciata*
- *Drosophila eugracilis*
- *Drosophila subobscura*

The application was prepared in accordance with Section 303EE of the *EPBC Act.* The applicant proposes to use these species to study the mechanism of genome evolution by examining gene expression and tissue anatomy. For this purpose, they have requested that the *Drosophila* spp. are not desexed or restricted to a single sex for import.

A risk assessment was undertaken to evaluate the likelihood of establishment and spread in Australia, and the potential consequences of these impacts on the Australian environment. Several factors were considered during the assessment, including, but not limited to, available scientific evidence, biological and ecological traits of the species, environmental conditions for establishment (current and potential distribution of the species), conservation status, and potential impacts in the event of the establishment or spread of the species.

The diet of vinegar flies consists of decaying organic matter and microbes, unlike the true fruit flies from the family Tephritidae that oviposit in living, healthy plant tissue. Vinegar flies and fruit flies should not be confused with each other as the later contains several spp. known to be highly invasive and destructive pests.

The 4 species of *Drosophila* have different distributions, with two species overlapping. None of the 4 species of *Drosophila* are considered pests, however, *D. subobscura* has been introduced to the west coast of Canada, the United States of America, Chile and Argentina. Published peer-reviewed literature suggests that these 4 species would not have any deleterious consequences for the Australian environment should they establish a viable wild population.

Under s 391(1) of the EPBC Act, the Minister must take account of the precautionary principle in making decisions. The precautionary principle is triggered by two threshold conditions: 1) a threat of serious or irreversible environmental damage (direct or indirect); and 2) scientific uncertainty as to the environmental damage. After critically examining and reviewing all available evidence and information, the department has determined that these 4 species do not pose a threat of serious irreversible damage to the Australian environment; they are not considered harmful to humans or other species and their impact on the environment is negligible (low). The department recommends listing the 4 species of *Drosophila* on Part 2 of the Live Import List with the import conditions of:

- Research only. High security facilities only.

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1 Introduction

1.1 Purpose of the proposed import

A research application was submitted to amend the Live Import List to include 4 species of vinegar fly (*Drosophila* spp.). The species were *D. azteca, D. bifasciata*, *D. eugracilis* and *D. subobscura*. The applicant aims to use these species to study the mechanism of genome evolution by examining their gene expression and tissue anatomy. For this purpose, the applicant has requested that the *Drosophila* spp. not be desexed or restricted to a single sex for import.

1.2 Background on the Act

Under section 303EC of the *Environment Protection and Biodiversity Conservation Act 1999* (the EPBC Act), the responsible Minister may amend the List of Specimens taken to be suitable for live import (the Live Import List) by, relevantly, including a specimen on the list. Section 303EB of the EPBC Act dictates two parts to the Live Import List:

- Part 1 comprises specimens that can be imported without a permit under the EPBC Act. Part 1 must not include any organism listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES); and
- Part 2 comprises specimens that require a permit under the EPBC Act to be imported. For each specimen included in Part 2 of the list there is to be a notation regarding whether the inclusion is subject to restrictions or conditions. Restrictions or conditions may relate to a quantitative limit, the circumstances of import, the source of the specimen or the circumstances in which it was taken. Additional conditions may also be applied when the permit for import is issued.

Before amending the Live Import List, the Minister must consult with appropriate state, territory and Commonwealth ministers and may consult such other persons or organisations as the Minister considers appropriate and consider a report assessing the potential environmental impacts of the proposed amendment (section 303EC of the EPBC Act). When submitting an application to the department to amend the Live Import List, all applicants are required to provide an accompanying draft assessment report that addresses specific terms of reference published on the department's website.

The department undertakes an assessment of the potential environmental impacts of the proposed amendment using the information in the applicant's draft report and any other sources of relevant information. The department also considers comments and information received through the public consultation process (including states and territories). The application and accompanying draft report for the proposed import of 4 species of vinegar flies (*Drosophila spp.*) was released for public comment between 12 December 2021 and 4 February 2022. This assessment report on the potential environmental impacts of listing the species was prepared in accordance with Section 303EE of the EPBC Act. Under Section 303EC(5)(a) the Minister must consider this report before amending the list.

2 Biology and Ecology

A general search for the 4 species' names, '*Drosophila subobscura, Drosophila bifasciata, Drosophila azteca, Drosophila eugracilis'*, was conducted using online search engines to locate information, including the Department of Climate Change, Energy, the Environment and Water (DCCEEW)'s OneSearch, Google Scholar, ResearchGate. Results were primarily focused on genetic and evolutionary research.

2.1 Taxonomy

Despite the extensive use of *Drosophila* in biological and genetic research, controversy remains for phylogenetic relationships and taxonomy. The *Drosophila* spp. assessed herein belong in the subgenus *Sophophora*, which is further divided into several species and subspecies groups. The species groups relating to the 4 *Drosophila* spp. include *obscura* and *melanogaster*. The species subgroups in *obscura* includes *affinis (D. azteca)* and *obscura (D. subobscura* and *D. bifasciata).* The species subgroup under *melanogaster* includes *eugracilis (D. eugracilis)* (Table 1).

Table 1 Taxonomy of *Drosophila* spp. assessed herein, including *D. azteca*, *D. bifasciata*, *D. subobscura* and *D. eugracilis*

Kingdom	Animalia		
Phylum	Arthropoda		
Class	Insecta		
Order	Diptera		
Family	Drosophilidae		
Genus	*Drosophila*		
Subgenus	*Sophophora*		
Species group	*Obscura*		*Melanogaster*
Species subgroup	*Affinis*	*Obscura*	*Eugracilis*
Species	*D. azteca* (Sturtevant & Dobzhansky 1936)	*D. subobscura* (Collin in Gordon 1936) *D. bifasciata* (Pomini 1940)	*D. eugracilis* (Bock & Wheeler 1972)

Sturtevant (1942) divided the *obscura* species group into two lineages, namely the *affinis* subgroup (exclusively New World species) and *obscura* subgroup (Old and New World species). Buzzati-Traverso and Scossiroli (1955) stated there were two distinct lineages of Nearctic species and several lineages of Palearctic species within the *obscura* subgroup (sensu Sturtevant 1942). The obscura species group is now divided into 5 subgroups: *affinis* and *pseudoobscura* (New World species) and *obscura*, *subobscura* and *microlabis* (Old World species). The *affinis* and *pseudoobscura* species groups are sister taxa. Old World lineages are considered basal to the New World species (Markow & O'Grady 2006; O'Grady & DeSalle 2018).

Disagreement exists on the placement of *Drosophila eugracilis*. Schawaroch (2002) places this species close to the melanogaster species subgroup. Kopp and True (2002) and Kopp (2006) suggest that *D. eugracilis* is basal to all other oriental subgroups and that the melanogaster species subgroup

is sister to the *suzukii-takahashii* lineage. The applicant states that *D. eugracilis* uniquely forms its own subgroup within the melanogaster species group and therefore will provide greater insights into the mechanism of speciation and genome evolution.

2.2 Description

The family Drosophilidae is a highly diverse, cosmopolitan family of flies that includes fruit flies and vinegar or pomace flies (Markow & O'Grady 2006). *Drosophila* is a genus of this family and should not be confused with the distantly related family, Tephritidae (true fruit flies), because the later are frugivorous and include several highly invasive and destructive pest species.

The 4 species of *Drosophila* assessed herein are described below. More detail is provided by Markow & O'Grady (2006). Little to no detailed information on the body weight and size of the 4 species is available, however most are a similar size and weight to *D. melanogaster* (Karan et al. 1998); adult females and males are between 2-3 mm (including wings) and weigh approximately 100-160 mg.

2.2.1 Drosophila azteca

Adults are dark in colour, but not uniformly black. The male abdomen is shiny black posteriorly, apically involuted and appears truncated. Males are thinner than females and have a dark spot at the tail of the abdomen. Small sex comb on basitarsus only, with 4 to 6 small teeth; acrostichal setulae in six rows; frons dull, dark brown; mesonotum pollinose, sometimes marked with 4 dark brown longitudinal bands (two inside and two outside dorsocentral rows); front not pollinose, shining; six or fewer prensisetae on surstylus (Figure 1; Markow & O'Grady 2006).

Figure 1 *D. azteca*



Source: BIO Photography Group, Biodiversity Institute of Ontario

2.2.2 Drosophila bifasciata

Adults are dark in colour and have red eyes. Males are thinner than females and have a dark spot on the tail of the abdomen (Figure 2). The tip of the aedeagus is sharply cut and the paraphyses are slender (Markow & O'Grady 2006).

Figure 2 *D. bifasciata*

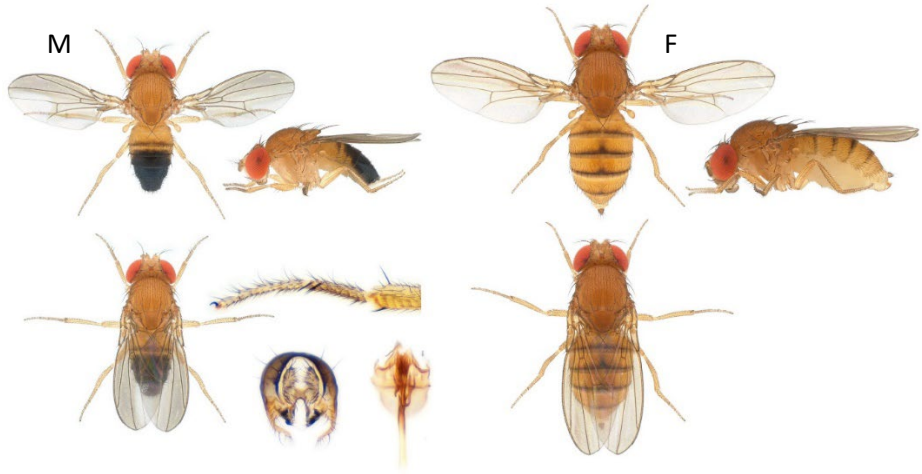


Source: BIO Photography Group, Biodiversity Institute of Ontario

2.2.3 Drosophila eugracilis

Adults are yellowish with yellow halters and legs and red eyes (Figure 3). Female very similar to *D. melanogaster* female; male abdomen shiny black posteriorly, apically involuted, appearing truncated; male fore metatarsus with 2 large distal bristles (Bock 1976). Their sex combs have only two strong setae (Markow & O'Grady 2006).

Figure 3 *D. eugracilis* male (M) and female (F)



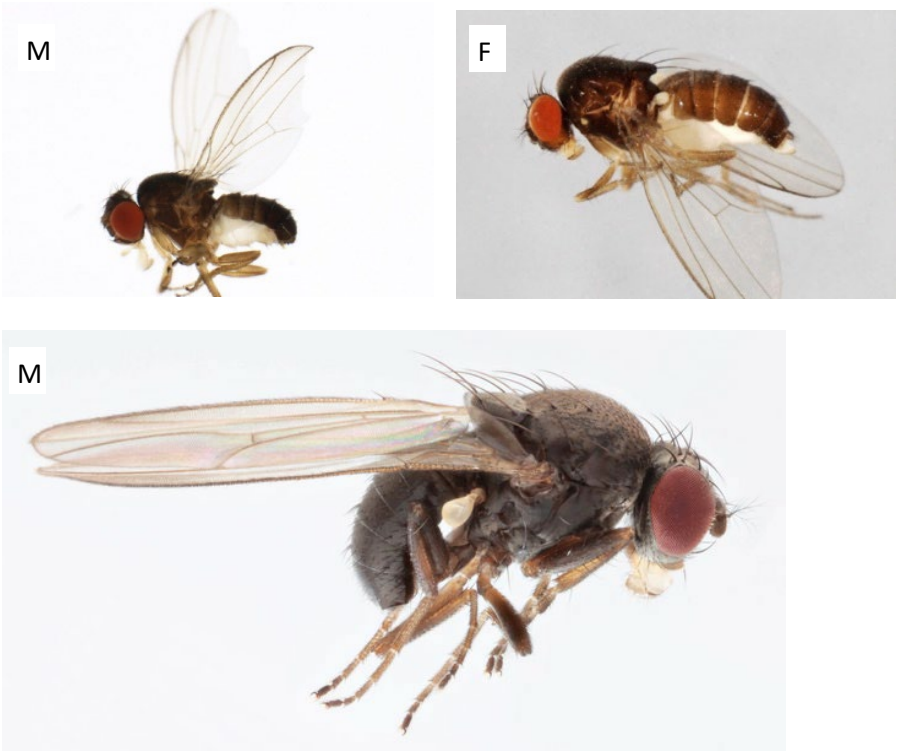
Source: Nicolas Gompel from i5k.nal.usda.gov/*Drosophila*_eugracilis

2.2.4 Drosophila subobscura

Adults are dark brown with yellow halters and legs, and red eyes (Figure 4). Males have a brown antenna, grey pollinose and brown dorsal surface of the thorax. The tergum (dorsal area excluding the head) is dark brown. There are three tarsal segments on the fore legs; the first segment is the longest and contains the proximal comb with 7-12 teeth; the second segment contains the distal

comb with 10-13 teeth. Females share the same characteristics as the males, except the tarsal combs. Males are thinner than females and have a darker spot at the tail of the abdomen.

Figure 4 *D. subobscura* male (M) and female (F)



Source: Malcolm Storey / Discover Life / v3.boldsystems.org / Çatal et al. 2021

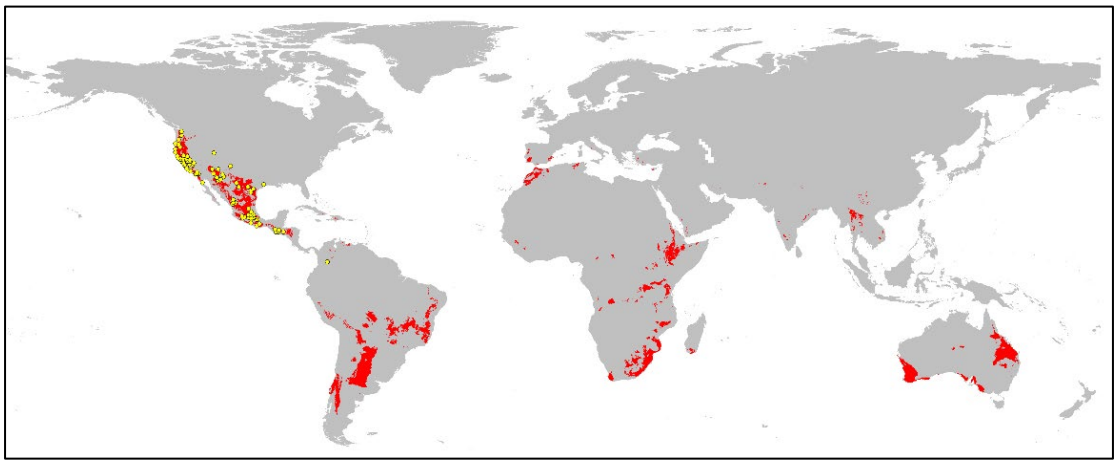
2.3 Geographic distribution

Table 2 Geographic distribution of the 4 *Drosophila* species assessed herein

Species	Geographic distribution
Drosophila azteca	Originating in Chilpancingo, Mexico, *D. azteca* is distributed throughout Mexico and Central America in the Nearctic and Neotropical regions (Moreteau et al. 2003). Predicted distribution of *D. azteca*, based on its current distribution and climatic conditions is shown in Figure 5.
Drosophila bifasciata	Originating in Kuusamo, Finland, *D. bifasciata* is Palearctic and widely distributed throughout Europe and Asia (Figure 6; Pomini 1940; Burla 1951; Markow & O'Grady 2006), in the Palearctic region. In Japan, it is found predominately in cool and mountainous regions but can also be found at low elevations in early summer (Moriwaki et al. 1952; 1953; 1954; 1967).
Drosophila eugracilis	*D. eugracilis* is distributed throughout Asia, southeast Asia and New Guinea, including India, China, Myanmar, Thailand, Cambodia, Malaysia, the Philippines, Borneo, Java, Sumatra and New Guinea (Figure 7; Achumi et al. 2016; Bock 1976; Gupta 1974; Jezovit et al. 2017; Markow & O'Grady 2006; Shao-liang et al. 2005; Takada & Momma 1975). There is some controversy as to its presence in Australia. Several databases, including GBIF (2022), ALA (2022), ABRS (2009) and CABI (2022), have no occurrence records in Australia. However, Bock and Wheeler (1972) and Bock (1976) reports *D. eugracilis* occurring in '*rain forests, Cairns area, north Queensland*'. Markow and O'Grady (2006), Brake and Bächli (2008), Takada & Momma (1975) and Jezovit et al. (2017) re-cite Bock (1976). No other accounts are given by Bock & Parsons (1977), McEvey and Bock (1982), McEvey (1982; 1983), Van Klinken

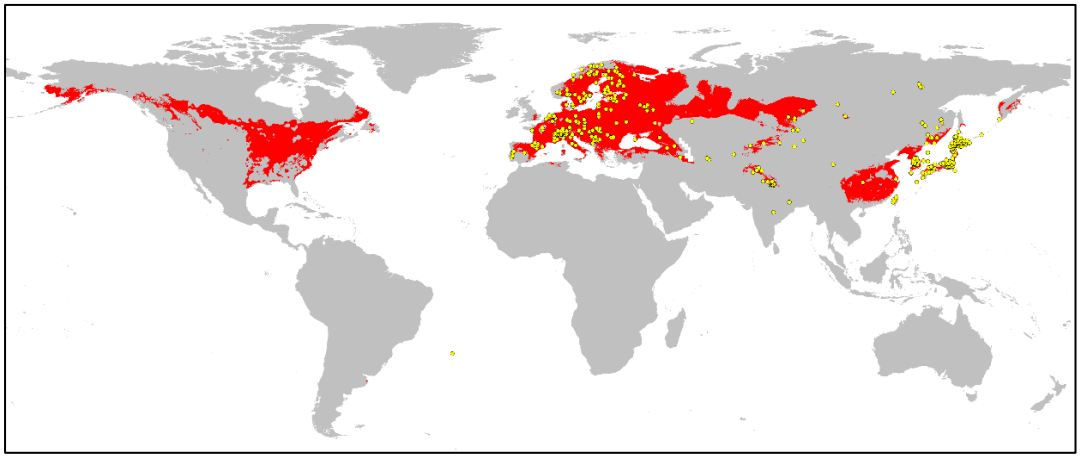
Species	Geographic distribution
	(2002), Mather (1955; 1956) or ADEER (2015). The status of *D. eugracilis* in Australia remains uncertain, but a restricted distribution is assumed based on Bock (1976) and further literature re-citations.
Drosophila subobscura	Originating in Montgenèvre, France, *D. subobscura* was considered an endemic Palearctic species that has spread from its traditional range into Neotropical and Nearctic regions. It is widely distributed in Europe, from Scandinavia south to the Mediterranean, and in North Africa and the Middle East as far east as Iran (Figure 8; Prevosti et al. 1989). It was introduced in the west coast of Canada, the United States of America, Chile and up the Atlantic coast of Argentina (Brncic et al. 1981; López 1985; Ayala et al. 1989). Specifically, in February 1978 it was detected in Puerto Montt, Chile (Brncic et al. 1981) and in 1982 in Port Townsend, Washington (Beckenbach & Prevosti 1986). Presently the species is detected in an area ranging from 29° to 53°S in Chile and from 35° to 55°N in western North America (Brncic et al. 1981; Ayala et al. 1989). Its geographical distribution has been well documented and studied; *D. subobscura* occurs in woodland, forest fringes, open fields (pasture) and moorland (Prevosti et al. 1989; Pascual et al. 1993; Noor et al. 2000). Buzzati-Traverso and Scossiroli (1955) state that this species can adapt to a wide variety of habitats. The genome of some Greek populations of *D. subobscura* has shown evidence of microgeographic variation, prompting a possibility that the species exhibits habitat choice (Krimbas & Alevizos 1973). However, no evidence suggests this species exhibits individual habitat choice, aligning with the fact that its well-studied inversion polymorphism is inflexible and slow to respond to the environment (Atkinson & Miller 1980).

Figure 5 Global distribution of *D. azteca* (yellow) and predicted distribution (red) based on suitable climatic conditions



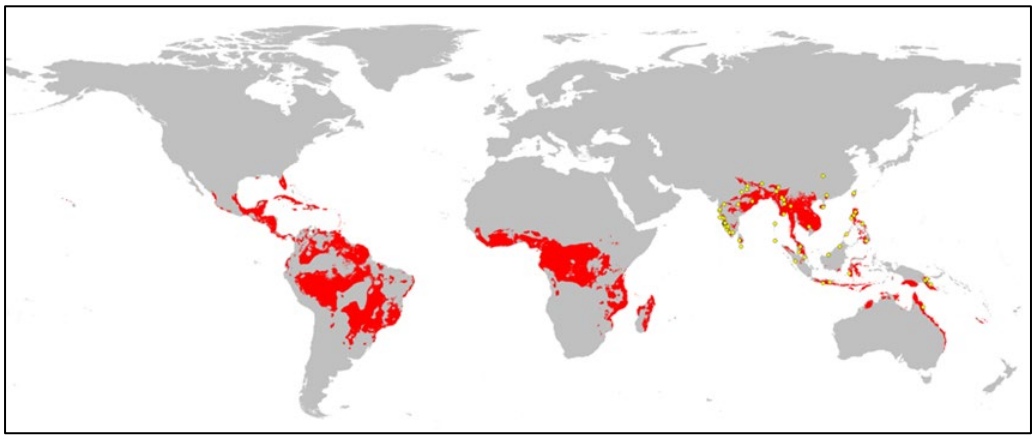
Source: http://evolution.ibmc.up.pt/

Figure 6 Global distribution of *D. bifasciata* (yellow) and predicted distribution (red) based on suitable climatic conditions



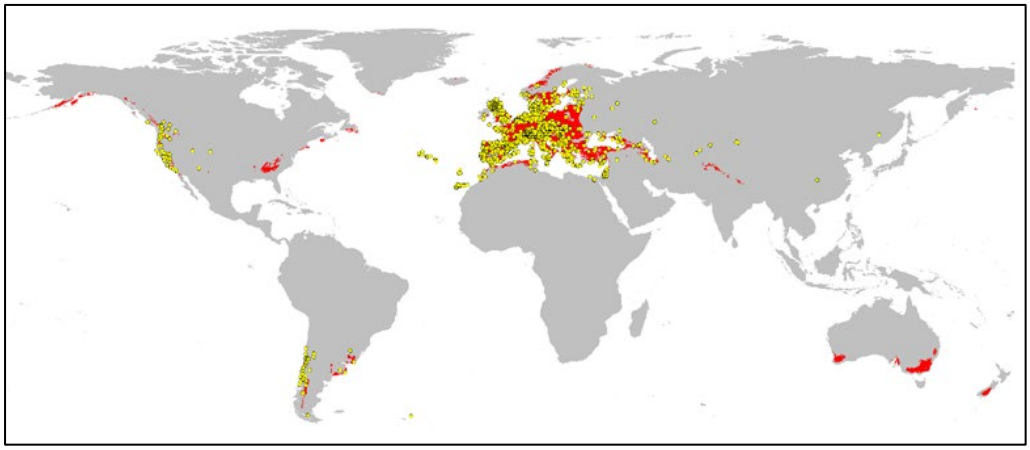
Source: http://evolution.ibmc.up.pt/

Figure 7 Global distribution of *D. eugracilis* (yellow) and predicted distribution (red) based on suitable climatic conditions



Source: http://evolution.ibmc.up.pt/

Figure 8 Global distribution of *D. subobscura* (yellow) and predicted distribution (red) based on suitable climatic conditions



Source: http://evolution.ibmc.up.pt/

2.4 Diet

The 4 species of *Drosophila* assessed herein are saprophagous. That is, that they feed on, or obtain nourishment from, decaying organic matter. This matter is often overripe and fallen fruit, dead and decaying plant matter, and microbes responsible for decomposition such as bacteria, moulds, and yeasts (Walsh et al. 2011). A major limiting resource for drosophilid population sizes is fruit availability (Döge et al. 2015). In the laboratory, drosophilids are often maintained on a diet of fermenting media (Markow & O'Grady 2006).

2.5 Reproductive biology

Courtship and mating have been extensively studied for *Drosophila* (Ewing 1983; Spieth 1974; Tomaru & Yamada 2011). *Drosophila* use a complex combination of visual, auditory, mechanosensory and chemosensory cues during courtship and mating (Bontonou & Wicker-Thomas 2014; Schneider et al. 2012b; Agrawal et al. 2014; Kohatsu & Yamamoto 2015; Ramdya et al. 2015; Duménil et al. 2016).

Courtship and mating are influenced by the presence of light with some species mating in darkness and light, while others only mate in light (Markow & O'Grady 2006). Several species have a narrow window of time during the day which they mate (Markow & O'Grady 2006). Of the 4 species assessed herein, *D. subobscura* are monandrous (females only mate once) and only mate in light as they rely on visual stimuli for courtship (Philip et al. 1944; Fisher et al. 2013; Smith 2005). They are unique, in that they do not produce a courtship song via wing vibrations like other species of Dipterans (Ewing & Bennet-Clark 1968). The male courtship ritual in *D. subobscura* is composed of discrete elements that include attempts to tap the female body, female-oriented proboscis extension, midleg swing, wing scissoring, bilateral wing extension in front of a female and extension of the proboscis which contacts with the proboscis of a female to offer a regurgitated drop of the male's crop components as a nuptial gift (Spieth 1974; Steele 1986; Immonen et al. 2009; Greenspan & Ferveur 2000).

Preventing production and exchange of nutritional gifts among *D. subobscura* has been shown to decrease male mating success and the number of eggs produced by females (Steele 1986). Other *Drosophila* species have been shown to 'sleep' and exhibit circadian cycles of 'arousal' and activity (Li et al. 2021; van Swinderen & Andretic 2003; Zimmerman et al. 2004). Barometric pressure also impacts mating activity in many *Drosophila* species. For example, some have been observed to show little interest in courtship during storms or stormy seasons during the year.

In the wild, *Drosophila* flies aggregate on decaying fruit where they mate and oviposit. Food odours and pheromones play a vital role in mate location (Lebreton et al. 2012). Development time varies, but are assumed to be similar, between the 4 species and depends on environmental factors such as temperature, breeding substrate and number of flies, however, it is usually between 40-65 days when the temperature range is 21-23^{o}C (Yoon et al. 1990). Males reach sexual maturity between 0-19 days after eclosion and females between 0-6 days after eclosion (Pitnick et al. 1995). The larval stage has three instars. Females can produce approximately 500-1000 eggs in a lifetime although current literature suggests this number is less in the wild (Markow & O'Grady 2006).

Resource availability, competition and climatic conditions affect the biology and longevity of drosophilids, for example, resource availability determines larval density and survivorship, water availability affects ovary development and temperature affects longevity (Yoon et al. 1990; Wheeler 1996; Papaj 2000). Predators of *Drosophila* spp. include other terrestrial invertebrates, such as ants, spiders, praying mantis, robber flies and staphylinid beetles (Parigi et al. 2019).

2.6 Environmental tolerances

Numerous studies have been conducted on *Drosophila* responses to abiotic conditions, including heat (Stratman & Markow 1998; Krebs & Loeschcke 1995; Loeschcke et al. 1994; Hoffmann et al. 2003; Kellermann et al. 2012b), cold (David et al. 1998; Gibert & Huey 2001; Kellermann et al. 2012a; Andersen et al. 2015; Bertoli et al. 2010) and desiccation (Gibbs & Matzkin 2001; Kellermann et al. 2012a; Matzkin et al. 2009; Harshman & Hoffmann 2000). Tolerances to these conditions are reliable indicators of geographic distribution (Kellermann et al. 2012b; Andersen et al. 2015). Goto et al. (1999) showed that rearing at specific temperatures for *D. bifasciata, D. obscura* and *D. subobscura* provided an increased resistance to the cold at 15°C*. D. subobscura* can be bred to increase its resistance at temperatures of 33.5°C when reared at higher temperatures either as pre-adult or adult (Maynard-Smith 1956). Generally, *Drosophila* are highly sensitive to low moisture and require greater than 70 per cent humidity for successful reproduction (Ashburner et al. 2005).

2.7 Hybridisation

D. subobscura, *D. bifasciata*, *D. azteca*, *D. eugracilis* exhibit hybrid dysgenesis, that is, when females and males from different species copulate, any resulting progeny is infertile. Sturtevant & Dobzhansky (1936) have successfully bred *D. azteca and D. athabasca* hybrids in a laboratory setting. Khadem & Krimbas (1991) have successfully bred *D. madeirensis* and *D. subobscura* hybrids, producing sterile males and fertile female hybrids. *D. subobscura* does not breed with any other species of obscura.

2.8 Social behaviour and grouping

Animals of the same species tend to congregate in proximity within a group or social aggregate, such as swarm, flock, school, or herd of birds, fish, locust, or groups of bacteria (Mogilner et al. 2003; Parrish & Edelstein-Keshet 1999).

Flies show a tendency to be attracted to one another and to congregate (Bolduc et al. 2010; Lof et al. 2008; Lof et al. 2009; Simon et al. 2012; Sun et al. 2020). This increased proximity is required prior to other, more complex behaviours, such as mating or aggression (Chen et al. 2002; Connolly & Tully 1998). Limited information is available on these behaviours for the 4 species assessed herein, however, behaviours of other *Drosophila* spp. are discussed.

Aggression serves in the acquisition or defence of resources such as food, shelter or access to mates. Fighting and territorial behaviour has been studied in common Drosophilids, such as *D. melanogaster* and *D. simulans* (Hoffmann 1987; Chen et al. 2002). Hoffman (1990) reported that isolated male *D. melanogaster* exhibited more aggressive behaviours and required less time to establish their territories than males held in groups, suggesting that social experience suppresses aggressiveness in flies. Similar observations have been reported for female *D. melanogaster,* where aggressive behaviours became apparent one day after emerging from the larva stage (eclosion) (Ueda & Kidokoro 2002).

3 Species status and conservation

A general search for the 4 species' names, '*Drosophila subobscura*, *Drosophila bifasciata*, *Drosophila azteca*, *Drosophila eugracilis*', was conducted using online search engines to locate information, including the Department of Climate Change, Energy, the Environment and Water (DCCEW's OneSearch, Google Scholar, ResearchGate. Results were primarily focused on genetic and evolutionary research.

3.1 National status

Only *D. eugracilis* is thought to be established in far north Queensland, Australia. In Tasmania, *D. eugracilis* is not listed on the Regulated Quarantine Pest list or Unwanted Quarantine Pest List. In Western Australia, it is an Unlisted – s14 pest under the Western Australian Organism List. No additional regulatory measures are apparent for other states and territories in Australia to limit spread from its reported Queensland distribution. The other 3 species of *Drosophila* are not present in Australia.

3.2 Related Live Import List listings

Drosophila melanogaster is listed on Part 1 of the Live Import List with no import conditions. Twenty other *Drosophila* species are listed on Part 2, all with import conditions (Table 3). Specimens listed on Part 1 of the Live Import List do not require an import permit. Specimens listed on Part 2 of the Live Import List require an import permit and may also have import conditions imposed under the *EPBC Act* and the *Biosecurity Act 2015.*

Table 3 Non-endemic species from the genus *Drosophila* on the Live Import List

Scientific Name	Listing	Common name(s)	Import conditions
Drosophila melanogaster	Part 1	Common Fruit Fly, Vinegar Fly, Pomace Fly	None.
Drosophila americana	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila ananassae	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila birchii	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Research only. High security facilities only.
Drosophila busckii	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila buzzatii	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila erecta	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila grimshawi	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila hydei	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.

Drosophila mauritiana	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila mojavensis	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila pachea	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila pseudoobscura	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila repleta	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila santomea	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila sechellia	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila simulans	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila teisseri	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila virilis	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila willistoni	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.
Drosophila yakuba	Part 2	Fruit Fly, Vinegar Fly, Pomace Fly	Eligible non-commercial purpose only, excluding household pets. High security facilities only.

3.3 Related threat category listings

None of the species are on listed on the:

- List of Invasive Alien Species of Union concern (the Union list)
- Global Invasive Species Database (GISD 2022)
- Global Register of Introduced and Invasive Species (GIIS 2022)
- Centre for Agriculture and Bioscience International (CABI 2022)
- European and Mediterranean Plant Protection Organization (Organisation Européenne et Méditerranéenne pour la Protection des Plantes; EPPO 2022)

It should be noted that spotted wing drosophila (*D. suzukii*) is listed in CABI (2022) and on the EPPO alert list; it is a fruit crop pest and a serious economic threat to a wide range of fruit crops and wild fruits. *D. suzukii* is an economically damaging pest because the females can infest thin-skinned fruits before harvest and the larvae destroy the fruit pulp by feeding. The species is endemic in Asia and was recorded as invasive in Hawaii (in 1980), California and in Europe (in 2008). Since 2008 it has spread rapidly throughout the temperate regions of North America and Europe, due to global trade and the initial lack of regulation over the spread of any Drosophila. This species has a high reproductive rate and short generation time; *D. suzukii* can theoretically have up to 13 generations per year, which may contribute towards its rapid spread. The Australian government Department of

Agriculture, Fisheries and Forestry Biosecurity (2013) has undertaken a Pest Risk Analysis for *D. suzukii*.

Conservation status

3.3.1 IUCN Red List of Threatened Species

None of the species are listed on the IUCN Red List.

3.3.2 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)

None of the species are CITES-listed.

3.3.3 Conservation of Migratory Species of Wild Animals (CMS)

None of the species are CMS-listed.

4 Risk Assessment

Invasive non-native species are a major driver of biodiversity loss worldwide and are particularly a threat to geographically and evolutionarily isolated ecosystems (e.g., islands like Australia). In the framework of the international movement of animals, it is important to analyse and assess both the risk of a non-native animal becoming invasive and the risk it poses to ecosystems. There are no accepted risk assessment models used to calculate the establishment risk of invertebrates in Australia. Bomford (2008) found that for vertebrates, the level of risk can be assumed in accordance with the 4 key factors of establishment success. Although these factors apply to vertebrates, they are used as a guide for this risk assessment.

The 4 key factors are:

- Propagule pressure: release of large numbers of specimens at different times and places
- Climate match: introduction to an area with a similar climate to the species' current range
- History of establishment elsewhere: previous successful establishment success
- Taxonomic group: belonging to a family or genus with a high establishment success

4.1.1 Propagule pressure

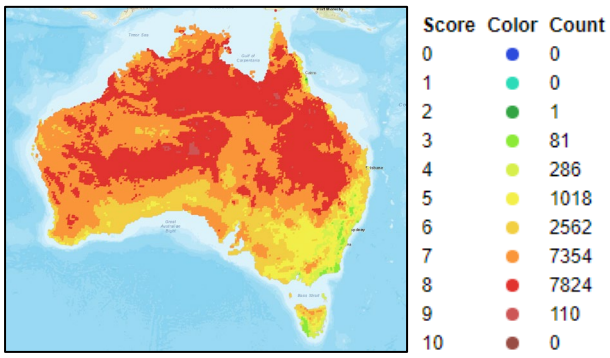
If listed on Part 2, the 4 species of *Drosophila* could only be imported for research purposes and require an import permit, which would limit the number of specimens imported into an Approved Arrangement (AA) accredited quarantine facility in Australia. The applicant states that they may then transfer the species to a PC2 laboratory, but as required by the Department of Agriculture, Forestry and Fisheries (DAFF) requirements, the applicant must first breed the specimens through one generation and inspect for hyperparasites (and other parasites, diseases, etc.) before release (if granted) from the AA facility. In the case of these 4 species of *Drosophila*, the applicant would only be allowed to remove *D. eugracilis* from the AA facility into the PC2 laboratory (if indeed it is already established in Australia). The other 3 species would be contained in an AA facility and additional specimens would be killed and disposed of. A one-off deliberate or accidental release is unlikely and multiple releases would be highly improbable. The likelihood of escape or deliberate release of specimens from an AA facility is negligible. These quarantine facilities are required to ensure that specimens are always secured, and their movement controlled to negate escape.

4.1.2 Climate match

Climatic factors are a major mechanism affecting invertebrate population sizes (Wolda 1978; Tidon 2006). Climatch v2.0 was used to predict the potential geographical distribution of the 4 species of *Drosophila* in Australia. Climatch matches climatic variables from the species' current distribution (source) to other regions in Australia (target). Predictions are presented in Figures 9-12. The higher the score (e.g., 10), the closer the match of climates between the source and target.

D. azteca is the most climatically suited of the 4 species, with the majority of mainland Australia, especially central and northern Australia (QLD, NT and WA) reflecting a suitable climatic match with its current distribution (Figure 9).

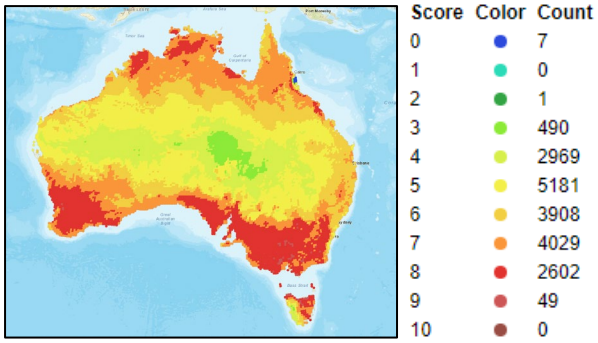
Figure 9 Climatch prediction on the potential distribution of *D.* azteca in Australia



Source: Climatch

D. bifasciata is most climatically suited to southern regions of Australia, including some of NSW, the whole of the ACT and Victoria, and southern SA and WA (Figure 10).

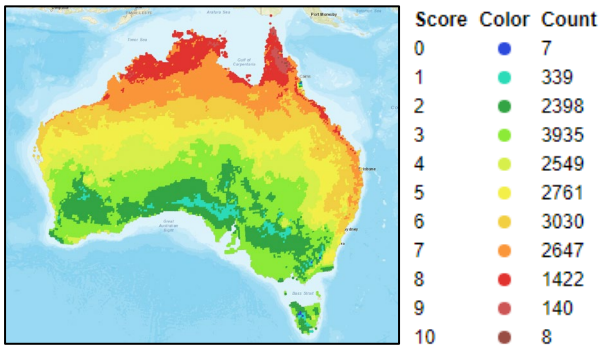
Figure 10 Climatch prediction on the potential distribution of *D.* bifasciata in Australia



Source: Climatch

D. eugracilis is thought to be present in QLD, and as predicted, it is most climatically suited to northern Australia including Cape York Peninsula in QLD and parts of NT and WA (Figure 11).

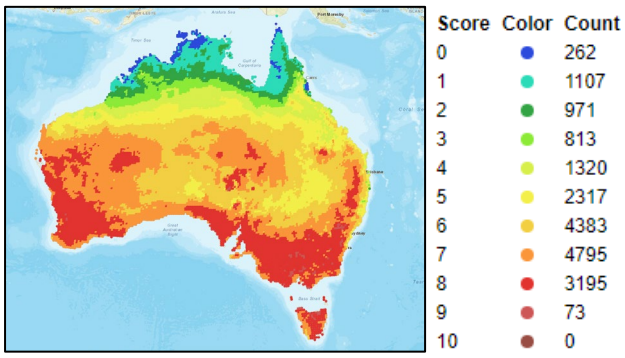
Figure 11 Climatch prediction on the potential distribution of *D.* eugracilis in Australia



Source: Climatch

D. suboscura is most climatically suited to southern parts of Australia, including Victoria, Tasmania and WA (Figure 12).

Figure 12 Climatch prediction on the potential distribution of *D.* subobscura in Australia



Source: Climatch

It should be noted that the annual average daily mean temperature of Australia has risen by 0.9 °C since 1910 (BOM & CSIRO 2020) and temperatures are projected to follow the global trend to increase by approximately 2–4 °C by 2100 (Arias et al. 2021). In addition, there is an increase in the fraction of Australia receiving summer (December to February, accumulated) rainfall that is above the 90th percentile (BOM & CSIRO 2020). A high proportion (greater than the 90th percentile) of annual rainfall has been increasing since the 1970s, thought significant regional variability exists, with the east coast region experiencing a significant decrease in extreme rain events since 1950 (BOM & CSIRO 2020). Species populations are responding to these changes by shifting their geographic distribution and assemblages (Lavergne at al. 2010; Parmesan & Yohe 2003; Parmesan 2006). Species distribution models may not reflect these rapid adaptive changes, yet this should be considered for future species' assessments (and for *Drosophila see* Xue et al. 2019; Sultana et al. 2020).

Drosophila (and other insects) respond more rapidly to climate change than plants and vertebrates due to their poikilothermic nature, short generation time and high reproductive rate. In addition to the expansion and/or contraction of geographic distribution, other responses to climate change may include changes in phenological patterns habitat selection and reproductive behaviour and output (Devi et al. 2019).

4.1.3 History of establishment elsewhere

D. subobscura has been recorded as being introduced into the west coast of Canada, the United States of America and Chile (Brncic et al. 1981; Ayala et al. 1989; Rozas et al. 1990; Pascual et al. 2007). This species is native to the Old World, where it ranges from North Africa to Scandinavia. These flies invaded Chile in the late 1970s and experienced a severe bottleneck in the process (only ~7–10 flies founded the South American population; Brncic et al. 1981; Rozas et al. 1990; Pascual et al. 2007). Nevertheless, this species quickly colonized a broad latitudinal range (Brncic et al. 1981; Prevosti et al. 1985). From Chile, a second propagule soon invaded North America (~100–150 effective founders, Pascual et al. 2007) and quickly colonized the west coast (Beckenbach & Prevosti 1986).

Successful invasions have been documented for other species of *Drosophila*, such as the spotted wing *drosophila* (*D. suzukii)*, and within the Drosophilidae family, such as the African fig fly (*Zaprionus indianus*) and (Brncic et al. 1981; Vilela 1999; Fernández Iriarte et al. 2009; Calabria et al. 2012; Cini et al. 2012; Asplen et al. 2015).

4.1.4 Taxonomic group

Successful invasions usually occur when a species is adaptive in response to climatic conditions and available resources, there is a high environmental similarity with its current geographic distribution, generalist species with r-strategy and high resistance to environmental stresses, such as desiccation and thermal stress (Dobzhansky 1965; Stanley et al. 1980; David & Tsacas 1981). Water availability, temperature, light and vegetation type are major factors determining the micro-distribution of invasive and non-invasive drosophilids (Powell 1997; Döge et al. 2015).

Two species, *D. suzukii* and *D. pulchrella*, are known to have serious deleterious effects on agricultural fruit crops (Capinera 2001; van der Linde et al. 2006; Hauser et al. 2009; Hauser 2011; Kwadha et al. 2021). They are considered pest species because they oviposit in healthy fruit as opposed to fruit that is damaged or overripe (Sasaki & Sato 1995; 1996; Mitsui et al. 2006). Oviposition causes physical damage to the fruit due to the prominent serrated ovipositor of the female, which provides access to secondary infection by insects and pathogens including fungi, yeasts and bacteria (De Camargo & Phaff 1957, Molina et al. 1974, Louise et al. 1996). Eggs develop into larvae within the fruit, causing it to become soft and rot rapidly, resulting in reduced crop yields and significant financial losses.

4.2 Consequences for the environment

It is important to consider consequences for the Australian environment in the potential event that these species may become established in the wild.

One consequence may be competition for resources with other species, especially other native Australian *Drosophila*. *D. eugracilis* is thought to be present in northern Australia where several *Drosophila* species are found, including *D. immigrans, D. sulfurigaster, D. rubida* and *D. pseudotetrachaeta,* to name a few (see Bock 1976 for more species accounts). It is unknown how these species interact and impact each other in the wild, if at all. Interestingly though, in mixed laboratory cultures, *D. azteca*, is described by Pascual et al. (2000) as being the worst competitor, probably due to a combination of factors such as longer developmental time, longer initiation of oviposition in the cultures, lower fecundity, lower carrying capacity.

The 4 species of *Drosophila* will likely have no significant impact on plants or agricultural crops, as they lay their eggs in already damaged or rotting fruit that would not be harvested or eaten and do not attack healthy, undamaged fruit. This is unlike other drosophilid flies (for example, *D. pulchrella, D. subpulchrella, D. suzukii*; DAWE 2020). There may be some negligible impacts on insects or other animals that feed on host plants due to a reduced availability of fruits.

There are no anticipated human health consequences. There is no evidence or history of any food-borne related illnesses. The 4 species of *Drosophila* do not exhibit aggressive behaviours or any behavioural traits that would harm humans or other species.

There may be some impact on insect or animal species that feed on host plants due to reduced availability of fruits through larval competition or highly damaged fruits. However, these consequences are mostly unknown.

In summary, the impacts of these 4 species of *Drosophila* on agricultural production and human health are considered negligible. This is because these species are not aggressive, are not known to

harbor any diseases that pose a risk to humans or other species and do not exhibit traits that indicate they could become so. Unlike the closely related *D. suzukii*, these 4 spp. do not feed on fresh fruit. Given the lack of information regarding possible consequences for the environment, a risk rating of unknown for the environmental consequences is appropriate.

4.3 Concerns raised during consultation

The first consultation round seeking comment on the applicant's draft Terms of Reference (ToR) report was undertaken from 12 December 2021 to 4 February 2022. A total of 3 responses were received. These responses are presented in Appendix A. In summary, issues raised from respondents included:

- Differentiating between the families Tephritidae and Drosophilidae (referring to the proposed species for import as 'vinegar flies', not 'fruit flies')
- Not addressing BICON requirements for *Drosophila* spp.
- Inadequate containment plan

These concerns were adequately addressed by the applicant and the final ToR report was updated to reflect these changes.

4.4 Risk mitigation

Specimens will be used for research purposes only and will be imported from specialised laboratories in Japan or the United States of America (as described by the applicant). Specimens will be initially imported into an AA facility. The applicant wishes to move the specimens into an PC2 facility, if possible. Specimens will be doubly contained during transportation in a plastic vial with a cotton plug within a larger container.

Additional measures have been installed in the AA and PC2 accredited facilities to minimise the risk of potential release of specimens. These include installing an anteroom, placing fly zappers in the anteroom and main laboratory, installing sealed windows, and covering air ventilation outlets with mesh to prevent escape of specimens.

Any surplus specimens will be killed either by freezing or by heating at 90^{o}C for one hour in doubly contained plastic bags, which are then autoclaved and incinerated, inside the AA accredited facility.

The import condition added to these species will ensure that additional imports of specimens will be for research only, in high security facilities only.

. Specimens will also be required to be contained in the AA and PC2 accredited facilities once imported into Australia.

5 Discussion

The risk of specimens escaping or being deliberately released into the wild in Australia is negligible, given the strict security measures that would be undertaken and conditions of import. Imported specimens would be required to remain in the AA and PC2 accredited facilities. These premises are audited by the Department of Agriculture, Fisheries and Forestry and must meet strict criteria to ensure that specimens are kept in a highly secure environment that prevents their escape. All surplus specimens will be appropriately treated and disposed of as part of the certification of the facility. Specimens will be for research purposes only, and for this reason, the applicant has requested that the specimens not be desexed or restricted to a single sex because the study requires material from intermediate stages in the *Drosophila* spp. life cycle. This request has been considered when compiling this assessment report.

Factors to be considered in the likelihood of establishment in Australia and subsequent impact include the availability of resources (especially decaying matter, such as overripe fruit), climatically suitable areas, reproductive strategy of the 4 *Drosophila* spp. and their potential for adaptation. For all 4 species, there are available resources, especially in northern Australia where fruit farms exist. Based on Climatch, parts of Australia are climatically suitable for all 4 species. *Drosophila* exhibit the *r* reproductive strategy and a vast body of literature supports the potential for their adaptation to Australian environmental stresses and conditions. The viable minimum number of individuals needed to establish a wild population is not known, though *D. subobscura* invaded Chile with only ~7–10 flies founding the South American population (Brncic et al. 1981; Rozas et al. 1990; Pascual et al. 2007). From Chile, a second propagule invaded North America with ~100–150 effective founders (Pascual et al. 2007). *D. eugracilis* is thought to be established in far north Queensland in Australia. To date, no detrimental impacts on the Australian environment have been recorded for this species. The other 3 species share a similar biology and ecology, and it is assumed that they too would have little to no detrimental impacts on the Australian environment.

6 Conclusion

The department has reviewed the application to amend the *List of Specimens Taken to be Suitable for Live Import (Live Import List)* to include the 4 species of *Drosophila* and undertaken a risk assessment. During this process, the department independently reviewed all available information on the 4 species of *Drosophila* and sought consultation from Commonwealth, state and territory agencies, the public and other experts, as is required under the EPBC Act.

The department concludes that these species pose a negligible (low) risk to the Australian environment. One of the species being assessed is thought to be established in Australia with no deleterious effects on the Australian environment. These 4 species are saprophytes that consume dead and decaying organic matter. They are not aggressive and do not pose any known risk to humans or other species of animals. However, they do exhibit an *r* strategy, can produce many offspring and have wide environmental tolerances that indicate a broad potential geographic range.

After critically examining and reviewing all available information, the department concludes that these species pose a negligible (low) risk to the Australian environment. The department recommends listing the 4 species of *Drosophila* on Part 2 of the Live Import List with the import conditions of:

- Research only. High security facilities only.

Appendix A: Comments received on the proposal to include 4 species of Vinegar Flies (*Drosophila* spp.) on the Live Import List

The applicant's draft assessment report on *Drosophila* spp. (4 species of vinegar flies) was posted on the DCCEEW website for consultation. The first consultation round was undertaken from 12 December 2021 to 4 February 2022. The applicant's final assessment report was sent to states and territories for a second consultation round from 23 August to 19 September 2022. A summary of the submissions and comments to be addressed are presented in Table 4. Please note that these comments are not endorsed by, nor reflect the position of, the department.

Table 4 Comments received during public consultation and response from applicant

Respondent	Major comments	Further information requested	Applicant response
The Victorian Government Department of Jobs, Precincts and Regions, Agriculture Victoria (Biosecurity & Agriculture Services)	• Current 'fruit fly' pest management programs in Australia refer to the family Tephritidae and include pest species such as Queensland fruit fly and Mediterranean fruit fly. Members of the family Tephritidae and *Drosophila* species in the family Drosophilidae, although both called "fruit flies", occupy very different habitats and have diverse life history and reproductive strategies. Technical information on pest management of *Drosophila* is not addressed in these fruit fly management programs. It would be helpful for the applicant to distinguish between high priority destructive agricultural pests in the Tephritidae family and the generally less damaging Drosophilidae in the opening comments of the application. • BICON requirements should be addressed. • The following statement in the 4 applications "In an extremely unlikely scenario where animals are found outside the AA- Accredited or the PC2 facilities, the facility manager will be informed and reinforce the risk mitigation procedure to all members of the laboratory who have access to these facilities" does not fully describe an adequate	**Refer** to the 4 *Drosophila* spp. as 'vinegar flies' instead of 'fruit flies' through the report to avoid confusion between members of the family Tephritidae and *Drosophila* species in the family Drosophilidae.	Applicant has updated terminology throughout the final report to 'vinegar flies' instead of 'fruit flies.'

Respondent	Major comments	Further information requested	Applicant response
	containment plan. Requests practices and physical measures outlined in formal procedural documents for containment of animals in the PC2 labs. • Requests more specific information on habitats, life histories, reproductive strategies, ecology, reproductive capacity, and potential impacts. • Request to indicate the numbers of animals for import, and over what time. • Request to indicate the fate of the contained research population after importation, e.g., destroyed, retained, etc.		
Member of the public,	• Each application is identical except for the species name and map of distributions. The application is poorly written, with many omissions. • No authorities were given for any of the 4 species and no references given to their descriptions. Also, italics are not used for the species name which is a basic error. • It is not clear why 4 similar species are needed nor is it explained why a study of their genomes are needed. What is most important is how will these species benefit Australia in understanding its current *Drosophila* fauna. • Concerns regarding secure housing in a PC2 quarantine facility. • We are not told how many individuals will be imported and if only one importation will be undertaken. • There is no mention that a reference series of male and female species will be deposited in at least two museums in Australia or that the bar codes of each species, and each importation of each species and be made available by deposition in GenBank. This is an essential requirement for every importation of any species alien to Australia. • A full morphological description of each species should be included. • Item 1 on the form is missing. Just to add a photograph of a male and female of each with a single short sentence explaining how males and females differ is totally inadequate. In this section accompanying each description there should be a full description of what is known of their biology and list of the regions/countries from where it has been recorded.	**Provide** specific information relating to the 4 species of *Drosophila* being assessed. **Provide** authorities for the 4 species of *Drosophila* being assessed. **Justify** the research aims and methodology for the 4 species of *Drosophila* being assessed. **Include** a morphological description for the 4 species of *Drosophila* being assessed. **Include** information on the number of individuals to be imported per import.	Additional measures have been installed at the AA-Accredited facility in the John Curtin School of Medical School at the Australian National University to minimize the risk of potential release of the specimen. 1. anteroom. 2. Fly zappers placed in the main room and anteroom. 3. Windows are sealed, and all ceiling vents covered by filters that prevent flies from escaping. More details of the procedure of the containment of the specimen during the experiments can be found in the following additional document (provided): GEN_SWP_001_Fly_Ovary_Dissection at AA quarantine-room.pdf The most recent audit report for the AA-Accredited quarantine facility (ANU N2548 N2549 and N2640 Audit Report.pdf) has also been provided. The experimental approach requires material from intermediate stages in the Drosophila life cycle (e.g. embryos and

Respondent	Major comments	Further information requested	Applicant response
			larvae) and, thus, must be maintained across generations. 50 to 100 adult flies will be imported each time. Once they are imported, animals will be bred in the AQIS-approved facility for 2-3 years until completion of the experiment. We are not aware of any Australian jurisdictions that have found Drosophila eugracilis harmful for the agriculture or the environment. Nor has importation of this species been prohibited
South Australian Government Department for Environment and Water	No comment.		

Glossary

CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora
IUCN – International Union for Conservation of Nature
SSC – Species Survival Commission
DCCEEW – Department of Climate Change, Energy, the Environment and Water
EPBC – Environment Protection and Biodiversity Conservation Act 1999
spp. – Species (plural)
sp. – Species (singular)
Live Import List – The List of Specimens taken to be suitable for live import

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