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# Host range testing of *Aphthona nonstriata* (Goeze) (Coleoptera: Chrysomelidae) for biological control of *Iris pseudacorus* in New Zealand

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# Summary

## Project and client

This report assesses the host range of the flea beetle, *Aphthona nonstriata* (Goeze) (Coleoptera: Chrysomelidae) as a prospective biocontrol agent for yellow flag iris, *Iris pseudacorus* L.) in New Zealand. The report was prepared by Manaaki Whenua – Landcare Research (MWLR) for the National Biocontrol Collective and MPI as part of the Multi-weed Biocontrol grant S3F20095.

## Objectives

To determine the suitability of the yellow flag iris flea beetle, *Aphthona nonstriata*, as a prospective biocontrol agent for yellow flag iris, *Iris pseudacorus* in New Zealand.

## Methods

- Adult *A. nonstriata* no-choice testing included 36 species in the plant family Iridaceae, two of which are endemic to New Zealand in the genus *Libertia*. Feeding by adult beetles was recorded and plants that supported feeding were maintained to monitor emergence of new, first-generation (F1) adults.
- Test species which supported complete development under no-choice conditions were subjected to choice tests.
- Host range testing was predominantly conducted at the Centre for Biological Control, Rhodes University, South Africa. Some testing was carried out at Vrije Universiteit in Brussels, Belgium, and testing of *Libertia* spp. was completed at The Bioeconomy Science Institute - Manaaki Whenua – Landcare Research Group's containment facility in Lincoln.

## Results

- Adult beetles attempted feeding on foliage of all test species under no-choice conditions, but feeding levels were high (> 41 %) only on species of the genus *Iris*.
- *Aphthona nonstriata* completed development to F1 adults on nine test species in both no-choice and choice tests. All nine species are in the genus *Iris*.
- No F1 adults developed on the *Libertia* species in either no-choice or choice settings.

## Conclusions

- The host range of *A. nonstriata* is restricted to the genus *Iris*.
- Within the genus *Iris*, development of *A. nonstriata* appeared to be restricted to certain subgenera.
- Some groups of cultivated irises, such as Louisiana irises (*I. ser. hexagonae*) and tall-bearded irises (*I. germanica*) were unsuitable for *A. nonstriata* development.
- Because *A. nonstriata* failed to develop on any test species outside the genus *Iris*, we expect the risk of direct damage by *A. nonstriata* to native New Zealand flora (including the native *Libertia* species) to be negligible, were it to be released in New Zealand as a biocontrol agent targeting *I. pseudacorus*.

- Potential damage and development of *A. nonstriata* on exotic ornamental irises can be mitigated or avoided through the application of conventional pest control methods, such as insecticides.

### **Recommendations**

We recommend the biological control programme targeting *I. pseudacorus* proceeds with an application to the Environmental Protection Authority (EPA) to release *A. nonstriata* as a biocontrol agent for *I. pseudacorus* in New Zealand.



# 1 Introduction

This report outlines the history of the biocontrol programme against yellow flag iris, *Iris pseudacorus* L. in New Zealand and globally, and presents the results from host range testing of the flea beetle *Aphthona nonstriata* (Goeze) (Coleoptera: Chrysomelidae) as a prospective biocontrol agent for *I. pseudacorus* in New Zealand. The report was prepared by the Bioeconomy Science Institute - Manaaki Whenua – Landcare Research Group or the National Biocontrol Collective and MPI as part of the Multi-weed Biocontrol grant S3F20095.

## 2 Background

### 2.1 Suitability of *Iris pseudacorus* as a target for biological control

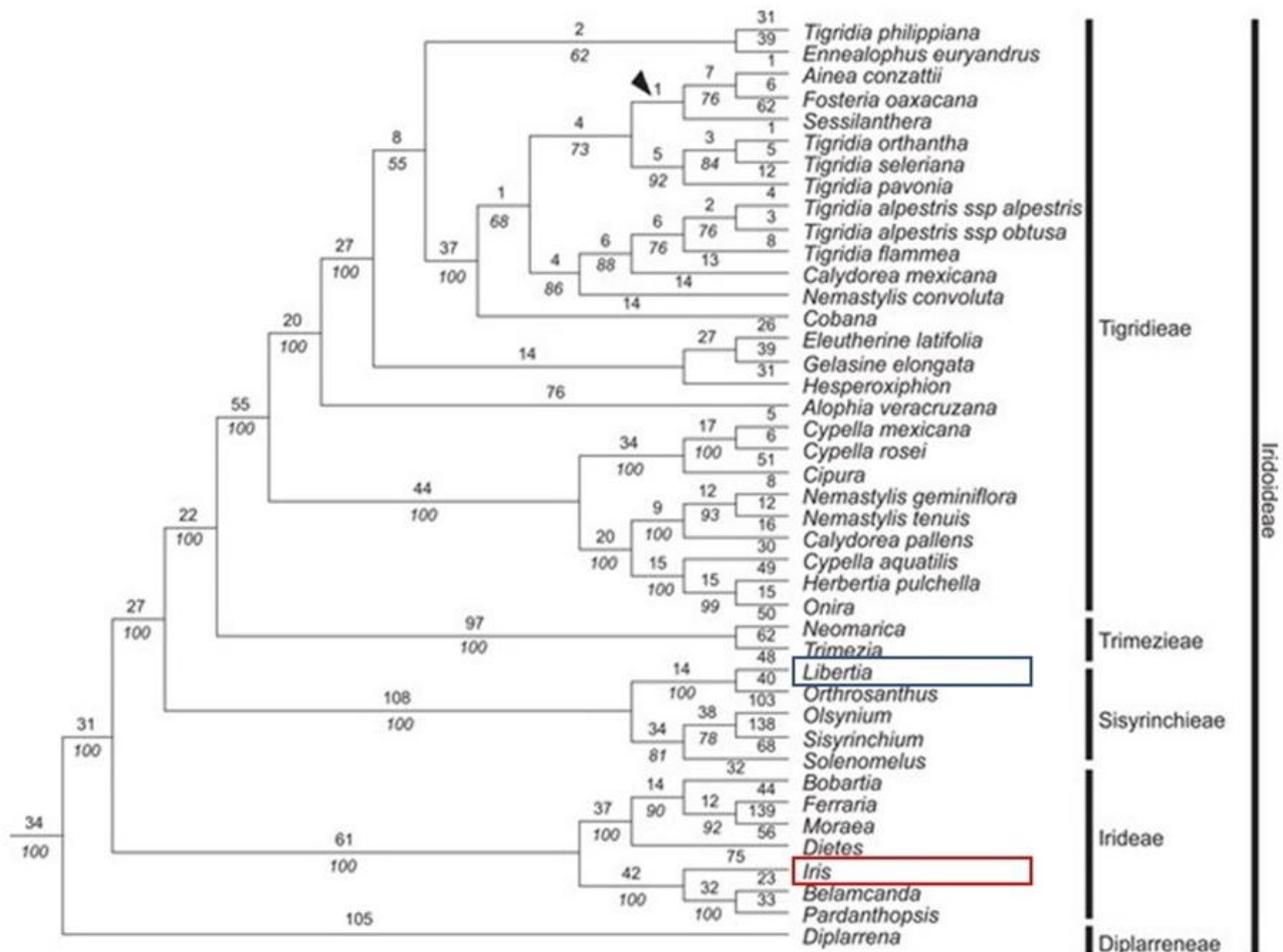
*Iris pseudacorus* L. (yellow flag iris) is a perennial wetland herb of the iris family Iridaceae (Figure 1; Figure 2). It is native to Europe, North Africa, the Mediterranean, and western Asia and has naturalised in many regions around the world, including New Zealand (Sutherland 1990; USDA-APHIS 2013; Kavak 2014), South Africa, Argentina and the USA. *Iris pseudacorus* has a wide distribution in New Zealand and is particularly weedy in the lower Waikato River catchment and along the Avon River in Christchurch. It is an unwanted organism in New Zealand under the Biosecurity Act (1993) and is also listed as a National Plant Pest Accord species, which prevents its sale and distribution in New Zealand. *Iris pseudacorus* threatens native flora in wetland and riparian habitats and is a problematic weed of wet pastures (Raven & Thomas 1970; Mopper et al. 2016). It can alter habitats through the formation of dense rhizome mats, which accumulate sediment and cause wetland areas to become drier (Sarver et al. 2008; King County Noxious Weed Program 2009). These rhizome mats are responsible for the displacement of native plant species (Lui et al. 2010; Morgan et al. 2019). *Iris pseudacorus* can clog waterways and irrigation ditches, which may affect farming and recreational activities (Jaca & Mkhize 2015; Morgan et al. 2019).

Manual control of *I. pseudacorus* is often ineffective, as small rhizome pieces will readily resprout (Tu 2003). Chemical control is the preferred current method for *I. pseudacorus* management in New Zealand, but requires repeat applications, is time, labour and cost intensive, and risks polluting waterways. Cost estimations (both labour and herbicide) of chemical control of *I. pseudacorus* in New Zealand is NZ\$100 to NZ\$340 per hectare when dealing with isolated patches, or as much as NZ\$1,350 per hectare or more when cover exceeds 40% (Wildland Consultants 2011). In 2019 MWLR was contracted by Waikato Regional Council to conduct a feasibility study to ascertain whether biocontrol of *I. pseudacorus* was achievable in New Zealand (McGrannachan & Barton 2019). That feasibility study recommended that biocontrol was a feasible option for the management of *I. pseudacorus* in New Zealand.



**Figure 1. Flowering plants of *Iris pseudacorus*.**

(Source: © Maureen Howard (CC-BY), retrieved from <https://inaturalist.nz/photos/450932503>).



**Figure 2. Phylogenetic relationships between tribes of the subfamily Iridioideae (family Iridaceae). The tribal classification is shown on the right. The red box highlights the genus containing *Iris pseudacorus*. The blue box highlights the genus *Libertia* which represents the only genus with native New Zealand species in the family Iridaceae and thus the closest relatives to *I. pseudacorus* within the New Zealand native flora.**

(Source: Adapted from Goldblatt et al. 2008.)

## 2.2 Biocontrol initiatives for *Iris pseudacorus*

No agents have been released anywhere in the world for biocontrol of *I. pseudacorus*. However, both New Zealand and South Africa have ongoing biocontrol programmes targeting *I. pseudacorus* and BSI-MWLR (New Zealand) and The Centre for Biological Control at Rhodes University (South Africa) are actively collaborating to identify and develop biocontrol agents. The Centre for Biological Control at Rhodes University also has a strong collaboration with scientists from The National Scientific and Technical Research Council (CONICET) and the Foundation for the Study of Invasive Species (FUEDEi) in Argentina. As the *I. pseudacorus* invasion is particularly bad in Argentina (Julie Coetzee, Biocontrol Researcher, pers. comm., 6 March 2019), it is hoped that this collaboration will result in the eventual release of biocontrol agents for *I. pseudacorus* there.

A preliminary survey within the native range (Belgium and northern Italy) was conducted by Minuti (2018) to identify and prioritise candidate biocontrol agents (arthropods only) for *I. pseudacorus* in South Africa. Three species were identified as potential biocontrol agents: i) the iris sawfly, *Rhadinoceraea micans* (Klug) (Hymenoptera: Tenthredinidae); ii) the iris seed weevil, *Mononychus*

*punctumalbum* (J.F.W. Herbst) (Coleoptera: Curculionidae); and iii) the iris flea beetle, *Aphthona nonstriata* (Goeze) (Coleoptera: Chrysomelidae). All three species were relatively common and abundant at surveyed sites in the native range and were prioritised due to their preference for hosts in the genus *Iris* and their potential to cause damage (Minuti 2018). *Rhadinoceraea micans* was observed to cause severe defoliation but was found on fewer plants than the other two species (Minuti 2018). Adult *M. punctumalbum* were observed mining petals and sepals on *I. pseudacorus* but were also recorded causing minor feeding damage on flowers of *Iris spuria* L. (subgenus *Limniris*) and *Iris germanica* L. (subgenus *Iris*). Individuals of this species were also observed to perforate (or create holes in) *I. pseudacorus* fruits, where eggs were laid that developed into seed-feeding larvae. *Aphthona nonstriata* adults were found to exclusively occupy and feed on *I. pseudacorus*, with a predisposition toward young leaf tips.

Of the three potential candidates, *A. nonstriata* was prioritised by researchers at Rhodes University, as it was highly abundant on *I. pseudacorus* and has a broad seasonal availability (Minuti 2018). Minuti (2018) undertook short-term adult feeding assessments (of cut vs intact leaves) on four plant species: i) *I. pseudacorus* (*Limniris*); ii) *I. sibirica* (*Limniris*); iii) *I. germanica* var. *Florentina* L. (*Iris*); and iv) *Typha latifolia* L. (bullrush). Results showed that *A. nonstriata* feeding rates were significantly higher on *I. pseudacorus* than on all other species on both cut and intact leaves (Minuti 2018). Adult survival rates on cut leaves of *I. pseudacorus* were significantly higher than on cut leaves of *T. latifolia* (intact leaves were not tested).

A biocontrol programme targeting *I. pseudacorus* in New Zealand began in late 2019, funded initially by Waikato Regional Council, and later by the National Biocontrol Collective and the Ministry for Primary Industries (MPI). *Aphthona nonstriata* was identified as a good first candidate agent to pursue for New Zealand, given that host testing for this species was already under way at Rhodes University (McGrannachan & Barton 2019).

Research by Rhodes University focused on host specificity tests of *A. nonstriata* in containment. The flea beetle was tested on several species in the genus *Dietes* (Family: Iridaceae) native to South Africa. South Africa does not have any native *Iris* species and the genus *Dietes* is the closest relative to *I. pseudacorus* in the native South African flora. Several ornamental *Iris* species and cultivars were also tested. The full results of the host range testing conducted by Rhodes University between December 2018 and September 2021 are outlined in Section 5 of this report. Efforts are currently underway to import the iris flower weevil *M. punctumalbum* into containment at Rhodes University to begin host specificity tests. However, we determined not to pursue *M. punctumalbum* as a biocontrol agent for New Zealand because of its wider host range and because it attacks flowers, which may draw opposition from iris growers.

The research in South Africa included a study of genetic diversity of *I. pseudacorus* within its introduced range in the Southern Hemisphere. Initially, it was believed that *I. pseudacorus* reproduction and dispersal were predominantly vegetative. However, research by Gaskin et al. (2016) revealed high levels of genetic diversity in introduced populations of *I. pseudacorus* in the western USA, suggesting reproduction and spread is largely via seed. Samples of *I. pseudacorus* from New Zealand were sent to Rhodes University for inclusion in the genetic analysis. That genetic diversity analysis for the New Zealand samples determined that our *I. pseudacorus* populations have high levels of genetic diversity, suggesting: i) that sexual reproduction, as opposed to vegetative reproduction, is the prevalent mode by which *I. pseudacorus* reproduces in New

Zealand; and ii) dispersion of seeds, particularly via waterways, is a key factor in the weed's spread here.

## 2.3 Overview of *Aphthona nonstriata*

### Taxonomy

Order	Coleoptera
Family	Chrysomelidae
Genus	<i>Aphthona</i>
Species	<i>Aphthona nonstriata</i> (Goeze)

*Aphthona nonstriata* (Coleoptera: Chrysomelidae) adults are moderately elongate and a dark metallic blue colour, with light brown legs and pale yellow to dark brown antennae (Minuti 2018) (Figure 3a). Specimens collected during native range surveys ranged in body length from 2.4–3 mm, with no sexual dimorphism observed (Minuti 2018). *Aphthona nonstriata* has one generation per year (Ellis 2025). The beetle is thought to hibernate over winter at the imago stage (the last stage an insect attains during its metamorphosis). It emerges from diapause (suspended development) with the onset of sprouting of *I. pseudacorus* new leaves (late March to early April in Europe). Upon emergence, adults undergo a preoviposition period in which they feed on young *I. pseudacorus* leaves, causing extensive damage. Adult feeding behaviour consists of linear bite patterns caused by lateral head movements while crawling forward (Figure 3b). This results in furrow-like damage between two adjacent leaf veins, leaving visible notches wherever insect feeding is paused (Figure 3c). Gregarious behaviour has been observed in adult beetles that group during the day to feed on a small number of leaves (Minuti 2018). Oviposition occurs a few centimetres below the soil surface, close to the plant rhizomes (Minuti 2018). Eggs are clear to pale yellow, oval, and 0.5–0.6 mm long (Figure 3d). Occasionally, eggs are laid together in a single cluster by several females. Larvae emerge between early May and late July (in Europe). Upon hatching, larvae burrow deeper into the soil where they perforate the plant rhizomes to feed on the rhizome interior. It is believed larvae go through 3 larval instars within the rhizomes (Minuti 2018). Third instar larvae are 2–3 mm long, with a cream-coloured body and light brown head capsule (Figure 3e). Upon completing larval development, the larvae leave the plant rhizomes and pupate in the soil. The new generation of adults (the F1) emerge from late July to early August in Europe and are believed to enter hibernation from October onwards, depending on the local temperature and photoperiod (Minuti 2018).

Despite a wide distribution, *I. pseudacorus* populations generally exist at low local population densities in its native range and are associated with high abundance and high levels of attack by *A. nonstriata* (Minuti 2018; Cantarelli 2024).

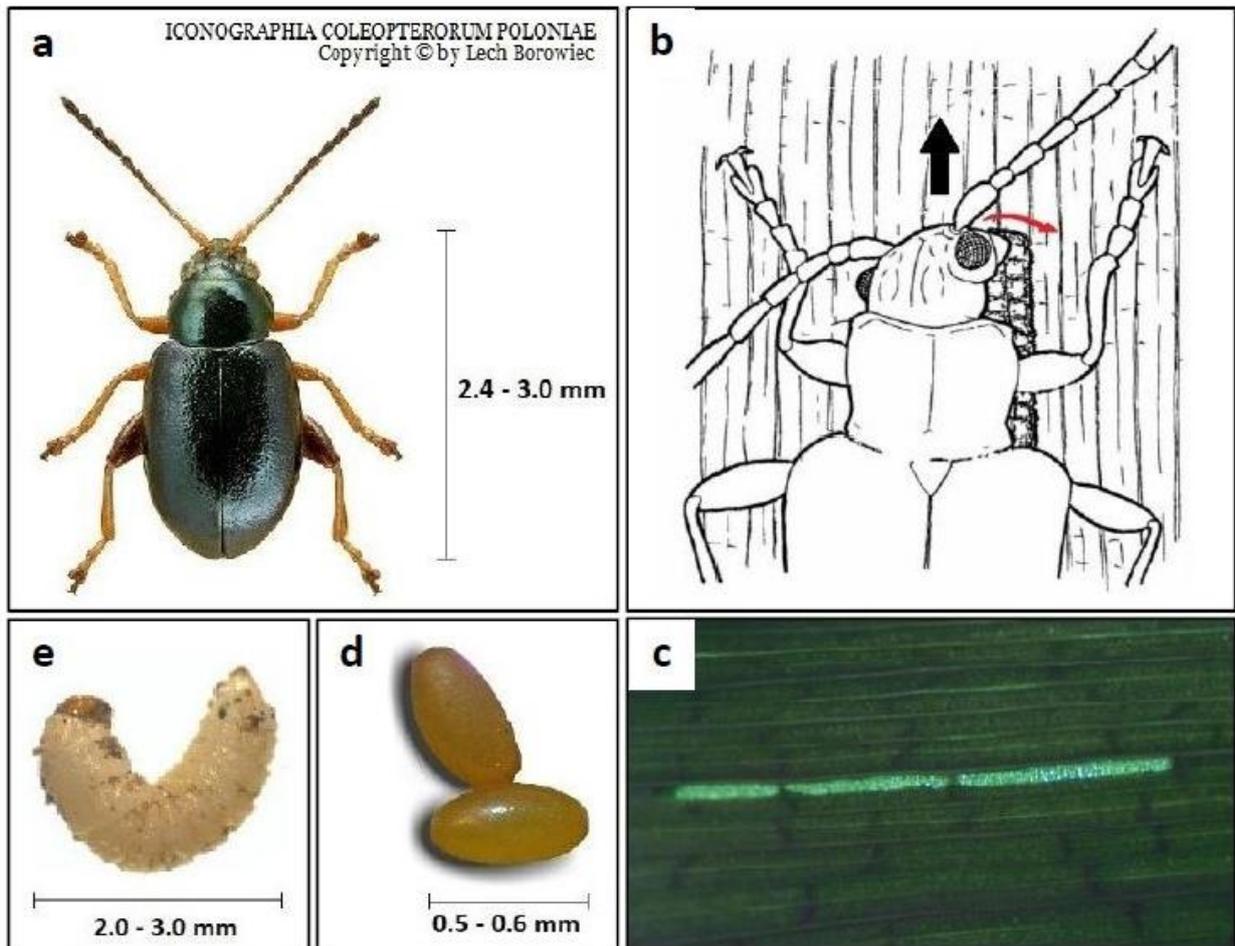


Figure 3. Biology and feeding behaviour of *Aphthona nonstriata* on *I. pseudacorus*: (a) Image of an adult *A. nonstriata*; (b) Dorsal view of the movement of the head (red arrow) and of the body (black arrow) of *A. nonstriata* while feeding; (c) Damage from *A. nonstriata* feeding on an *Iris pseudacorus* leaf; (d) *A. nonstriata* eggs, with size ranges; (e) Picture of 3rd instar *A. nonstriata* larva.

(Sources: a) Adapted from Bienkowski 2010 and b) reproduced from Minuti 2018, with permission.)

### 3 Objectives

To determine the suitability of the yellow flag iris flea beetle, *Aphthona nonstriata*, as a biocontrol agent for yellow flag iris, *Iris pseudacorus* in New Zealand.

## 4 Methods

### 4.1 Selection of plant species for host range testing

In 2020, the Centre for Biological Control at Rhodes University was subcontracted by MWLR to conduct host range testing of *A. nonstriata* on six Iridaceae species present in New Zealand (Table 1). This was the most cost-effective and efficient approach because host range testing had already begun, and they had a healthy colony of *A. nonstriata* in containment.

Four of the selected species were *Iris* species of the subgenus *Limniris*, to which *I. pseudacorus* belongs. These species were *I. spuria*, *I. orientalis*, *I. laevigata*, and *I. foetidissima*. The first three species are cultivated as ornamentals in New Zealand, and *I. foetidissima* is considered an environmental weed (McAlpine & Howell 2024).

The additional two species are the nearest relatives to *I. pseudacorus* in the New Zealand flora, in the genus *Libertia* (Figure 2). Eight of the nine native *Libertia* species are endemic to New Zealand, and their conservation status ranges from 'Not Threatened' to 'Nationally Critical' (de Lange et al. 2024). There are also four exotic species of *Libertia* present in New Zealand. These two species, *L. ixioides* (G. Forst.) Spreng. and *L. peregrinans* Cockayne & Allan, were selected for testing to represent New Zealand's most closely related plant taxa. Both species are endemic to New Zealand, have wide distributions throughout the country, and were easy to source. Plants were sourced from Trees for Canterbury Nursery, Christchurch. Only these two *Libertia* species were tested; three of the remaining *Libertia* species are Nationally Critical or Nationally Uncommon with restricted distributions (*L. cranwelliae*, *L. edgariae*, *L. flaccidifolia*) and *L. grandiflora*, *L. micrantha* and *L. mooreae* could not be sourced from local nurseries. The final *Libertia* species - *Libertia ixioides* x *L. peregrinans* - is a little-known hybrid of the two tested *Libertia* species recorded from North-West Nelson.

At least 20 exotic genera in the plant family Iridaceae are represented in New Zealand's introduced flora, either as naturalised species or casual aliens (Schönberger et al. 2018). Exotic genera in the Iridaceae with high species representation in the New Zealand flora include *Gladiolus* L., *Iris* L., *Sisyrinchium* L. and *Watsonia* Mill. (Schönberger et al. 2018). New Zealand also has several *Iris* species and cultivars that are commonly used as ornamentals, including (but not limited to) *I. germanica*, *I. innominata*, *I. orientalis* and *I. spuria*. These are all rhizomatous species, and all of them except for *I. germanica* are in the same subgenus as *I. pseudacorus*, which is the subgenus '*Limniris*'. *Iris germanica* is in the closely related subgenus '*Iris*'.

**Table 1. List of Iridaceae species included in host range testing to determine the suitability of *Aphthona nonstriata* for release in New Zealand**

Species	New Zealand (NZ) status <sup>1</sup>	Reason for inclusion in host range testing
<i>Iris foetidissima</i>	Exotic. Environmental weed	Found throughout NZ; Same subgenus ( <i>Limniris</i> ) as <i>I. pseudacorus</i>
<i>Iris laevigata</i>	Exotic. Approved species; Naturalised	Used as ornamental; Same subgenus ( <i>Limniris</i> ) as <i>I. pseudacorus</i>
<i>Iris orientalis</i>	Exotic. Approved species; Naturalised	Used as ornamental; Same subgenus ( <i>Limniris</i> ) as <i>I. pseudacorus</i>
<i>Iris spuria</i>	Exotic. Approved species; Naturalised	Used as ornamental; Same subgenus ( <i>Limniris</i> ) as <i>I. pseudacorus</i>
<i>Libertia ixioides</i>	Endemic; Not Threatened	Distributed throughout NZ; represents the closest native relative genus to <i>I. pseudacorus</i> in the New Zealand flora.
<i>Libertia peregrinans</i>	Endemic; Threatened – Nationally Vulnerable	Distributed throughout NZ; represents the closest native relative genus to <i>I. pseudacorus</i> in the New Zealand flora.

*Iris laevigata*, *I. orientalis* and *I. spuria* were sourced from nurseries in South Africa for inclusion in their host range testing. *Iris foetidissima* was sourced from Europe and host range testing of this species was conducted in a containment facility at Vrije Universiteit in Brussels, Belgium.

The two *Libertia* species were intended to be tested at Rhodes University but following several unsuccessful attempts to propagate them in containment in South Africa, the testing was conducted in New Zealand. In January 2024, 34 adult *A. nonstriata* beetles were imported into the containment facility in Lincoln, and a colony was successfully initiated. Host range testing on *Libertia* species was completed in February 2025.

<sup>1</sup> Sources: de Lange et al. (2024); New Zealand Plant Conservation Network (2024); The New Zealand Iris Society (Inc) (2024)

## 4.2 Host range testing protocol

The protocols for host range testing of *A. nonstriata* in South Africa, Belgium and New Zealand were standardised as much as was feasible. No-choice tests were conducted for each test species, and further choice tests conducted on those test species where F1 generations developed. However, *Libertia* species underwent both no-choice and choice tests irrespective of F1 development, because some feeding by the original (F0) adults was observed on the foliage under no-choice conditions. A full list of the test species is presented in Table 2 in Section 5. The number of replicates and recording of beetle damage differed between South Africa, Belgium and New Zealand and the methodology for each region is outlined below. Across all regions, damage was assessed from leaves only.

*South Africa:* Three to 6 (generally 5) replicates were conducted for each test species. Intact plants (1 to 2) were caged separately (no-choice tests) or together with *I. pseudacorus* control plants (choice tests). No-choice tests were conducted simultaneously with 2 to 3 replicates of *I. pseudacorus* per test species as controls (caged separately to test species). Adult feeding damage during no-choice tests was recorded for test plants as the mean percentage damage relative to damage on corresponding control plants. Damage was categorised as 'Low' (0%– 20%), 'Moderate' (21%– 40%) or 'High' ( $\geq 41\%$ ). Each replicate was assigned the midpoint percentage of its damage category, and these were averaged for each test species as well as for *I. pseudacorus*, to generate an overall damage category score at the species/cultivar level.

*Belgium:* Two to 4 (generally 4) replicates were conducted for each test species for no-choice tests, while 7 replicates were used for *I. pseudacorus* in no-choice test (and these were not paired with test species as controls). For choice tests, 3 replicates were used (where 1 replicate = 1 test species plant + 1 *I. pseudacorus* plant). Adult feeding damage during no-choice tests was recorded for test plants as the mean percentage damage relative to the mean damage on the 7 replicates of *I. pseudacorus*. Damage was categorised as 'Low' (0%–20%), 'Moderate' (21%– 40%) or 'High' ( $\geq 41\%$ ). Because *I. pseudacorus* control plants were unpaired with test species, and instead the 7 plants of *I. pseudacorus* served as controls for all no-choice tests, we used a weighted mean of 25.04 for the number of F1 adults on *I. pseudacorus* (Table 2). This weighted mean was calculated as the total number ( $M$ ) of adults that emerged from *I. pseudacorus* ( $n = 651$ ) divided by the number of *I. pseudacorus* replicates ( $n = 7$ ), and then multiplied by the weighted fraction of *I. pseudacorus* replicates ( $n = 7$ ) to test species replicates ( $n = 26$ ), thus:

$$\frac{\text{Total adults from } I. \text{pseudacorus}}{n \text{ } I. \text{pseudacorus replicates}} \times \frac{n \text{ } I. \text{pseudacorus replicates}}{n \text{ Test species replicates}} = \text{Weighted mean}$$

$$\frac{651}{7} \times \frac{7}{26} = 25.04$$

*New Zealand:* Between 10 and 11 replicates were conducted for *L. ixioides* and *L. peregrinans*, respectively, and 14 for *I. pseudacorus* in no-choice tests, while 12 replicates were conducted for each species in choice tests. Generally, an *I. pseudacorus* replicate was run concurrently with one plant each of *L. ixioides* and *L. peregrinans*. Adult feeding damage during no-choice tests was recorded for test *Libertia* plants as the mean percentage damage relative to damage on paired *I. pseudacorus* control plants. Damage was categorised as 'Low' (0%– 0 %), 'Moderate' (21%–40%) or 'High' ( $\geq 41\%$ ).

From 10 to 20 *A. nonstriata* individuals were released on each test plant. The flea beetle is difficult to sex visually, so copulating pairs were isolated before releasing on plants, to ensure as close to an even sex ratio as possible. Young adults (i.e. at 1–2 weeks after emergence) were used where possible to avoid beetle death during testing. Insects were left on plants for 1–2 weeks, after which they were removed. After beetle removal, test species in choice tests were also removed and placed into cages separate to the *I. pseudacorus* control plants. These cages were then monitored for 8 weeks to see if F1 adults emerged from the plants. Containment room conditions were regulated, with temperature set to  $25 \pm 1$  °C, humidity set to  $65\% \pm 5\%$  and photoperiod at 16:8 (light:dark) hours.

### 4.3 Statistical analyses

A Kruskal-Wallis test was performed to determine if there was a significant difference in the mean number of *A. nonstriata* adults produced on non-*Iris* test species compared to *I. pseudacorus* controls, in no-choice tests. Two Kruskal-Wallis tests (one for no-choice and one for choice tests) were performed to compare the number of adults produced on both *L. ixioides* and *L. peregrinans* to *I. pseudacorus* controls. A *post-hoc* Dunn test was implemented to confirm that significant differences were between *I. pseudacorus* and both *Libertia* species (rather than between the two *Libertia* species). A final Kruskal-Wallis test was conducted to ascertain if a significant difference in mean number of *A. nonstriata* adult emergence existed between *I. pseudacorus* control plants and all *Iris* test species/cultivars in no-choice tests. An independent t-test was performed to determine if there was a significant difference in the mean number of *A. nonstriata* produced on test species/cultivars compared to *I. pseudacorus* controls in choice tests. Statistical analyses were performed in R version 4.4.1 (R Core Team 2025).

## 5 Results

### 5.1 No-choice tests

A total of 36 Iridaceae species and/or cultivars were included in no-choice testing of *A. nonstriata* in South Africa, Belgium, and New Zealand (Table 2). Of these, 20 (55.56 %) exhibited low ( $\leq 20\%$ ) mean relative adult feeding damage, 9 (25%) showed moderate (21%–40%) mean relative feeding damage and 7 (19.44%) exhibited high (41%–60%) mean relative damage. All species suffering high levels of adult feeding damage were from the genus *Iris* and all but one – *I. ensata* – supported complete larval development to adults. Mean relative adult feeding damage to New Zealand *Libertia* plants ranged from 0%– 55% of the damage observed on *I. pseudacorus* plants but was on average low for *L. peregrinans* (19.33% mean relative damage) and moderate for *L. ixioides* (36.10% mean relative damage; Figure 4).

**Table 2. List of species in the family Iridaceae (in alphabetic order) used in no-choice host range testing of *A. nonstriata***

Test species	Cultivar <sup>a</sup>	Country <sup>b</sup>	No. reps	Damage category <sup>c</sup>	Mean No. F1 adults <sup>d</sup>	Mean No. F1 adults <i>I. pseudacorus</i> <sup>e</sup>
<i>Aristea ecklonii</i> Baker	NA	South Africa	5	L	0	8.33
<i>Carex pendula</i> Huds.	NA	Belgium	4	L	0	25.04
<i>Crocsmia</i> sp. L.	NA	Belgium	2	L	0	25.04
<i>Dierama igneum</i> Klatt	NA	South Africa	3	M	0	15
<i>Dietes bicolor</i> (Steud) Sweet ex Klatt	NA	South Africa	5	L	0	31.67
<i>Dietes grandiflora</i> N.E.Br.	NA	South Africa	5	L	0	26.20
<i>Dietes iridiodes</i> (L.) Sweet	NA	South Africa	5	L	0	5.67
<i>Ferraria crispa</i> Burm.	NA	South Africa	3	L	0	33
<i>Gladiolus grandiflorus</i> Andrews	NA	South Africa	3	L	0	11
<i>Gladiolus</i> sp. Tourn. ex L.	NA	Belgium	4	L	0	25.04
<i>Hesperantha coccinea</i> (Backh. & Harv.) Goldblatt & J.C.Manning	NA	South Africa	3	L	0	10
<i>Iris confusa</i> Sealy	NA	South Africa	5	H	6.40	7.67
<i>Iris domestica</i> (L.) Goldblatt & Mabb.	NA	South Africa	5	H	19.60	3.67
<i>Iris ensata</i> Thunb.	NA	Belgium	4	H	0	25.04
<i>Iris foetidissima</i> L.	NA	Belgium	4	M	39.75	25.04
<i>Iris germanica</i> Sm.	'Garden Blue'	Belgium	4	M	0	25.04
<i>Iris germanica</i> Sm.	'Gold Kist'	South Africa	3	M	0	12.5
<i>Iris germanica</i> Sm.	'Irish Coffee'	South Africa	3	L	0	11.5
<i>Iris laevigata</i> Fisch.	'June Lake'	South Africa	5	H	14.20	57
<i>Iris laevigata</i> Fisch.	'Regal Open'	South Africa	5	M	41.40	40
<i>Iris orientalis</i> Wood-Mason	NA	South Africa	5	H	52.80	24
<i>Iris ser.</i> Hexagonae Diels	'Annie's Song'	South Africa	3	M	0	9
<i>Iris ser.</i> Hexagonae Diels	'Louisiana'	South Africa	5	L	0	16.67
<i>Iris ser.</i> Hexagonae Diels	'Louisiana for Dad'	South Africa	3	L	0	9.50
<i>Iris sibirica</i> L.	'Flight'	South Africa	3	M	36.33	38.50
<i>Iris sibirica</i> L.	NA	Belgium	4	M	0	25.04
<i>Iris spuria</i> Bertol.	'Archie Owen'	South Africa	3	H	20	13
<i>Iris spuria</i> Bertol.	'Destination'	South Africa	5	H	8	14
<i>Libertia ixiooides</i> (G.Forst.) Spreng.	NA	New Zealand	10	M	0	56.54
<i>Libertia peregrinans</i> Cockayne & Allan	NA	New Zealand	10	L	0	56.54
<i>Moraea huttonii</i> (Baker) Oberm.	NA	South Africa	4	L	0	10
<i>Moraea reticulata</i> Goldblatt	NA	South Africa	4	L	0	10.50
<i>Neomarica gracilis</i> (Herb.) Sprague	NA	South Africa	5	L	0	23.33
<i>Watsonia angusta</i> Ker Gawl.	NA	South Africa	5	L	0	9.67
<i>Watsonia borbonica</i> (Pourr.) Goldblatt	NA	South Africa	5	L	0	23.33
<i>Watsonia pillansii</i> L. Bolus	NA	South Africa	5	L	0	10.50

<sup>a</sup> Where applicable, a cultivar name is provided.

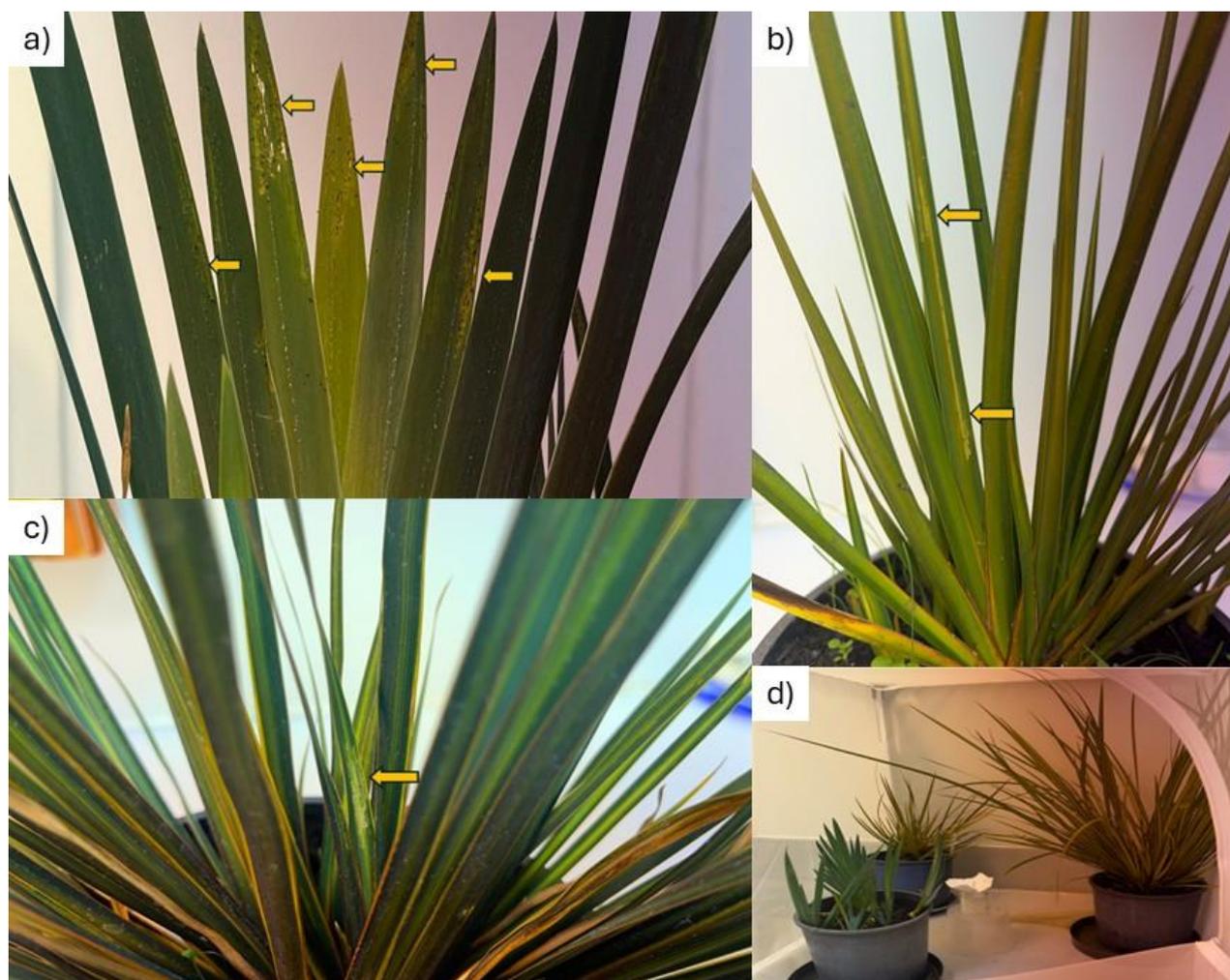
<sup>b</sup> In which of the three participating countries the species was tested.

<sup>c</sup> Adult feeding damage categories were: low (L = 0%-20%); medium (M = 21%-40%); high (H = 41%-60%). All percentages represent feeding proportion on the test species relative to feeding on the control species *I. pseudacorus*.

Categories presented here are the mean categories for the test species, calculated from midpoint percentages by replicate within species.

<sup>d</sup> Per replicate (rep) of the test species/cultivar.

<sup>e</sup> Mean number of adults emerging from the paired *I. pseudacorus* control replicates. Note: for species tested in Belgium, the number presented for *I. pseudacorus* is the weighted mean (refer to the Methods, Section 4, for Belgium tests for further details).



**Figure 4. Adult *Aphthona nonstriata* feeding damage after one week during choice tests on a) *Iris pseudacorus*, b) *Libertia peregrinans*, c) *Libertia ixiodes*. d) *Aphthona nonstriata* adult choice test set-up. (Left-back: *Libertia peregrinans*, left-front: *Iris pseudacorus*, right: *Libertia ixiodes*). Yellow arrows indicate areas of feeding damage.**

Adults successfully emerged from 9 species/cultivars from the genus *Iris*, including *I. confusa*, *I. domestica*, *I. foetidissima*, *I. laevigata* 'June Lake', *I. laevigata* 'Regal Open', *I. orientalis*, *I. sibirica* 'Flight', *I. spuria* 'Archie Owen', and *I. spuria* 'Destination'. The mean number of F1 adults emerging from these species ranged from 6.40 (*I. confusa*) to 52.80 (*I. orientalis*) (Table 2). Adult emergence occurred on all corresponding *I. pseudacorus* replicates and mean numbers of F1 adults ranged from 3.67–57.00. The mean number of *A. nonstriata* adults produced on *I. pseudacorus* was not significantly different to that of the *Iris* test species ( $\chi^2 = 3.19$ ,  $df = 1$ ,  $p = 0.07$ ; Figure 5). No adults completed development on species outside the genus *Iris*, including the two *Libertia* species. Mean adult emergence was significantly higher in *I. pseudacorus* compared to non-*Iris* species ( $\chi^2 =$

23.17, df = 1,  $p = <0.001$ ; Figure 6). The number of adults produced on *I. pseudacorus* was also significantly higher than that of both *Libertia* species (Kruskal-Wallis test:  $\chi^2 = 29.50$ , df = 1,  $p = <0.001$ ; Dunn test: adjusted  $p = <0.001$ ; Figure 7a).

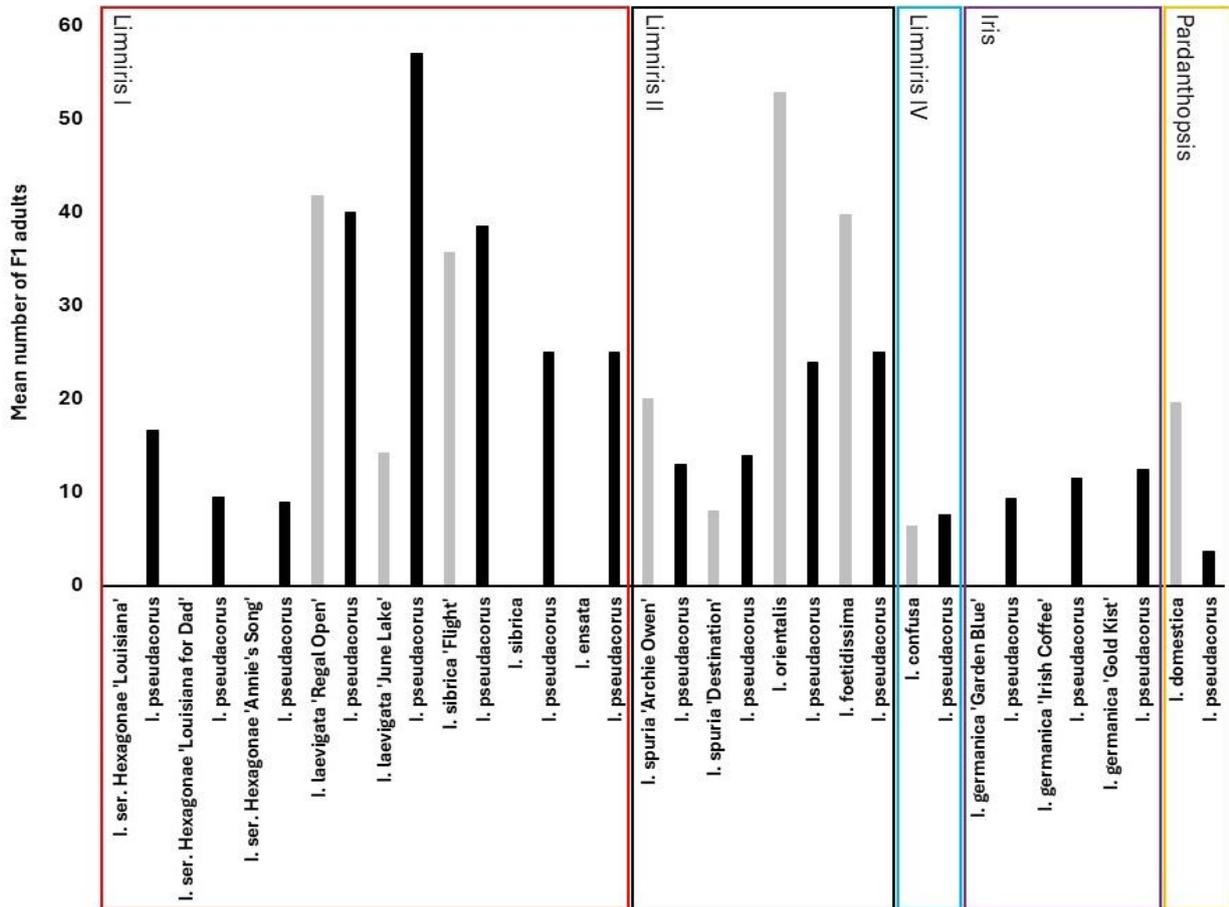


Figure 5. Mean number of *A. nonstriata* adults produced on *Iris* test species/cultivars (grey bars) and their corresponding *I. pseudacorus* controls (black bars). Coloured boxes indicate the *Iris* subgenus that *Iris* test species/cultivars belong to. Subgenera are ordered left to right by their relatedness to *I. pseudacorus* (*I. pseudacorus* belongs to subgenus Limniris I).

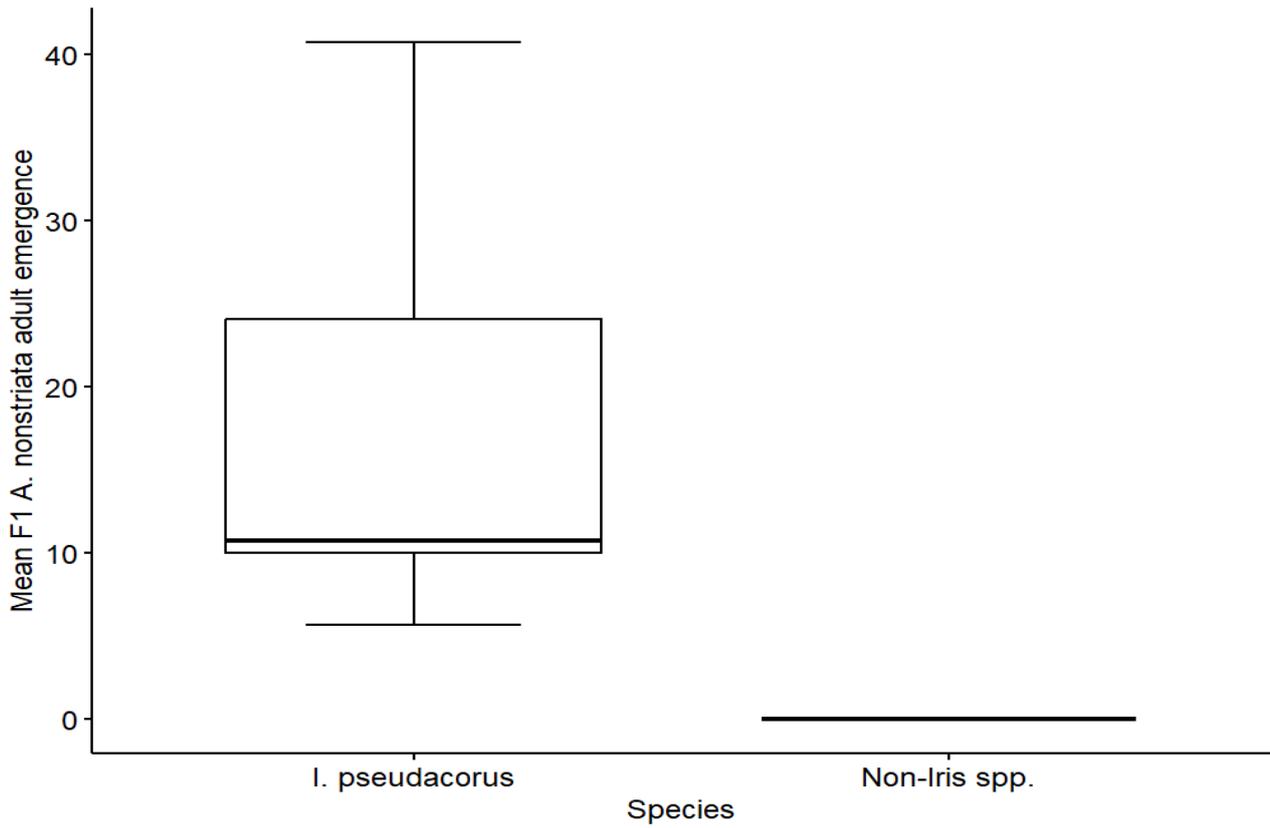


Figure 6. Mean number of *A. nonstriata* adults produced on non-*Iris* test species and *I. pseudacorus* control plants in no-choice tests.

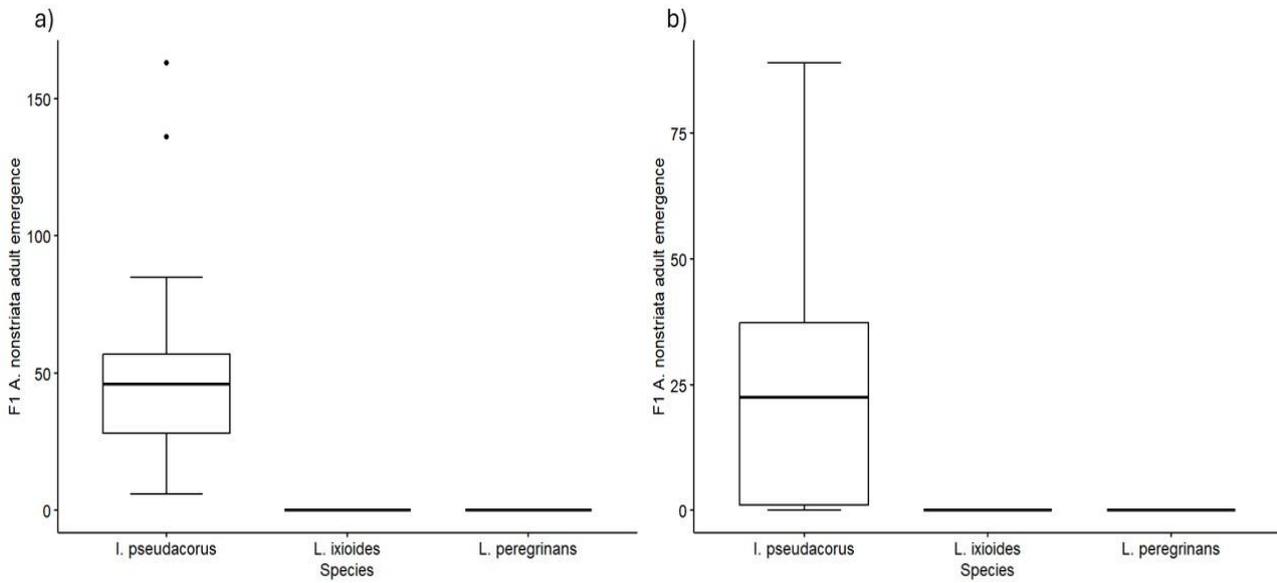


Figure 7. Number of *A. nonstriata* adults produced on *L. ixiooides*, *L. peregrinans*, and *I. pseudacorus* in a) no-choice tests and b) choice tests.

## 5.2 Choice tests

The 9 species/cultivars from the genus *Iris* on which *A. nonstriata* larvae successfully completed development in no-choice tests also supported successful complete larval development in choice tests. The mean number of adults produced from these species ranged from 4.60 (*I. confusa*) to 61.60 (*I. orientalis*) (Table 3). Adult emergence on *I. pseudacorus* replicates and mean number of adults ranged from 11.60 to 41.30. No larvae completed development on the two *Libertia* species tested and corresponding *I. pseudacorus* controls had significantly higher adult development (Kruskal-Wallis test:  $\chi^2 = 26.01$ ,  $df = 2$ ,  $p = < 0.001$ ; Dunn test: adjusted  $p = < 0.001$ ; Figure 7b). The mean number of *A. nonstriata* adults produced on *I. pseudacorus* was higher but not significantly different than the mean number produced on *Iris* test species/cultivars (two sample t-test;  $t = -1.15$ ;  $df = 20$ ;  $p = 0.26$ ; Figure 8). Three species – *I. laevigata* 'Regal Open', *I. orientalis*, and *I. spuria* 'Archie Owen' had higher mean number of adults produced than corresponding *I. pseudacorus* controls (Table 3). Notably, the mean number of adults eclosing on *I. orientalis* was almost three times the mean number from the corresponding *I. pseudacorus* replicate.

**Table 3. List of species in the family Iridaceae (in alphabetic order) used in choice host range testing of *A. nonstriata***

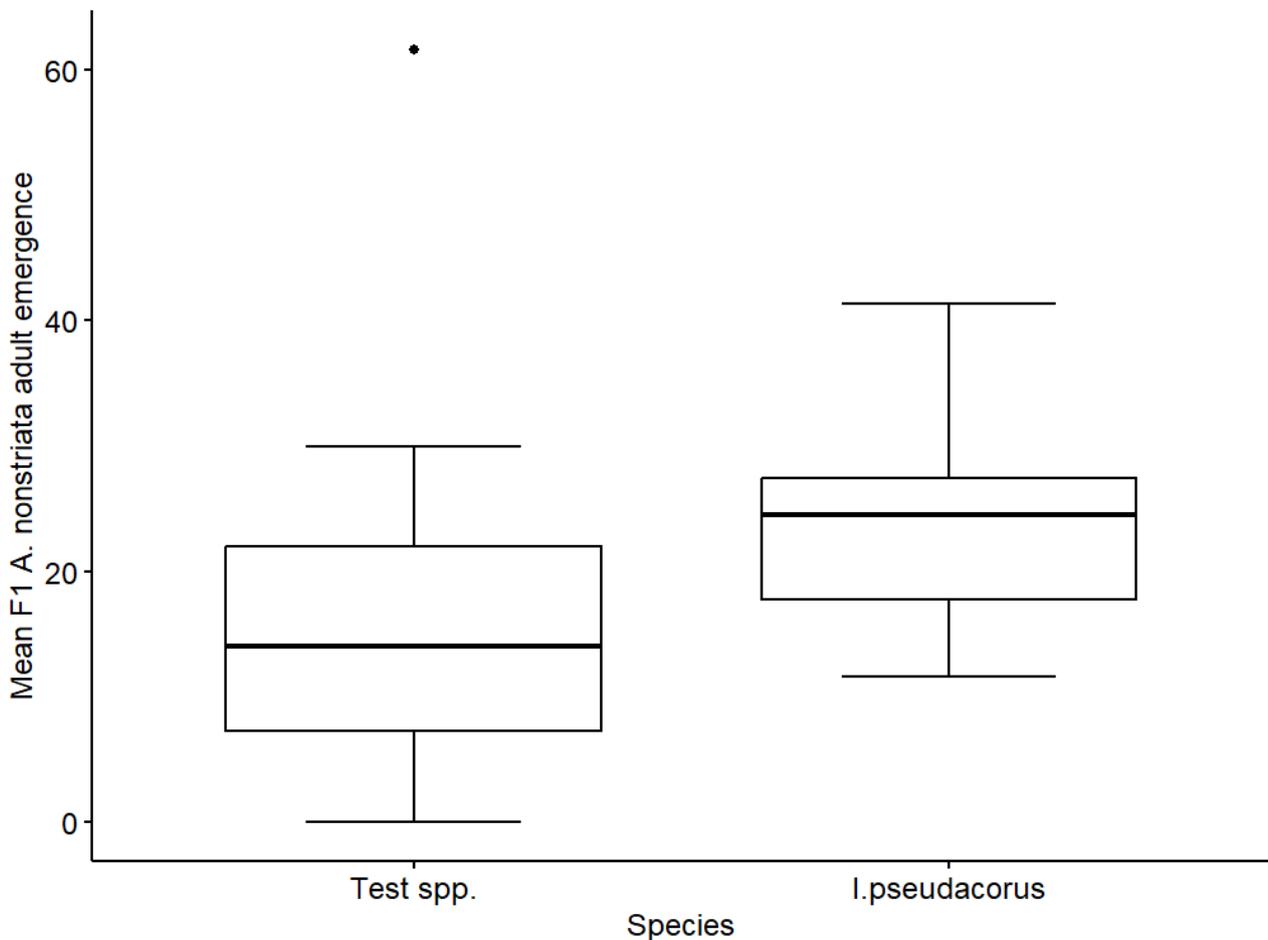
Test species	Cultivar <sup>a</sup>	Country <sup>b</sup>	No. reps	Mean No. F1 adults <sup>c</sup>	Mean No. F1 adults <i>I. pseudacorus</i> <sup>d</sup>
<i>Iris confusa</i> Sealy	NA	South Africa	5	4.60	13.20
<i>Iris domestica</i> (L.) Goldblatt & Mabb.	NA	South Africa	4	14.25	24.50
<i>Iris foetidissima</i> L.	NA	Belgium	3	10.30	41.30
<i>Iris laevigata</i> Fisch.	June lake	South Africa	5	10	15.20
<i>Iris laevigata</i> Fisch.	Regal open	South Africa	5	14	11.60
<i>Iris orientalis</i> Wood-Mason	NA	South Africa	5	61.60	24.40
<i>Iris sibirica</i> L.	Flight	South Africa	3	30	32.33
<i>Iris spuria</i> Bertol.	Archie owen	South Africa	3	22	20.33
<i>Iris spuria</i> Bertol.	Destination	South Africa	5	22	25.60
<i>Libertia ixioides</i> (G.Forst.) Spreng.	NA	New Zealand	12	0	27.42
<i>Libertia peregrinans</i> Cockayne & Allan	NA	New Zealand	12	0	27.42

<sup>a</sup> Where applicable, a cultivar name is provided.

<sup>b</sup> In which of the three participating countries the species was tested.

<sup>c</sup> Per replicate (rep) of the test species/cultivar.

<sup>d</sup> Mean number of adults emerging from the paired *I. pseudacorus* control.



**Figure 8. Mean number of *A. nonstriata* adults produced on *Iris* test species/cultivars and *I. pseudacorus* control plants in choice tests.**

## 6 Conclusions

The results of host range testing of *Aphthona nonstriata* on 36 Iridaceae species indicated that the flea beetle is restricted to development within the genus *Iris* and that even within the genus *Iris*, *A. nonstriata* is likely to be restricted to certain lineages. No-choice tests showed that a large proportion of Iridaceae species tested (44.12%) experienced only low levels of adult feeding. Those species that exhibited higher levels of adult feeding damage were restricted to the genus *Iris* (Table 2) which is not represented in the native flora of New Zealand. Some of these *Iris* species/cultivars are valued cultivated ornamental plants and could be at risk of attack. However, feedback from iris growers and nurseries in Europe revealed only one report of attack by *A. nonstriata* to *Iris* cultivars from a nursery in Germany. The nursery respondent reported that the damage associated with the flea beetle was uncommon and easily treated with available insecticides. Furthermore, an economic risk assessment estimating the costs and benefits of *A. nonstriata* biocontrol in New Zealand showed annual non-target attack costs of \$8.2K in 20245 – 1.1% of the predicted annual benefits from biocontrol (Fowler et al. 2025).

The genus *Iris* contains many species in several subgenera and sections. *Iris pseudacorus* belongs to the subgenus *Limniris I* (also known as the 'core *Limniris*'; Crespo 2011; Figure 9). Cultivars of *Iris sibirica* and *I. laevigata* which proved to be suitable hosts for larval development in testing, also belong to *Limniris I*. Interestingly, two cultivars of *I. sibirica* were tested, with one, *I. sibirica* 'Flight', supporting development to adults in both no-choice and choice tests while the other, an unknown cultivar of *I. sibirica*, did not. The other species of *Limniris I* tested was *I. ensata*, which also supported no adult development but exhibited the highest rate of adult feeding damage of all test species. 6 other *Iris* species/cultivars supported adult development. Five of them belong to two other *Iris* subgenera: *Limniris II* (*I. spuria* 'Archie Owen', *I. spuria* 'Destination', *I. orientalis*, *I. foetidissima*) and *Limniris IV* (*I. confusa*) (Crespo 2011; Figure 9). The sixth species – *I. domestica* – belongs to the subgenus *Pardanthropsis* and is the most distantly related to *I. pseudacorus* of the 9 species of *Iris* which supported F1 adult development (Crespo 2011; Figure 10). Given these results, it is possible that *A. nonstriata* development to the adult stage is possible on *Iris* species belonging to these subgenera. However, because New Zealand does not have any native species within the genus *Iris*, the risk of any direct feeding damage of the beetle on native flora is expected to be negligible and transient.

Despite *A. nonstriata* successfully developing on some species/cultivars of *Iris*, other *Iris* species/cultivars supported no development. Louisiana irises (*I. ser. hexagonae*) and tall-bearded irises (*I. germanica*), did not support *A. nonstriata* development, meaning these two groups of irises are highly unlikely to support populations of the flea beetle in the wild.

Despite some adult feeding occurring on foliage of test species outside the genus *Iris*, no adult development occurred on any of these species. This includes the two endemic New Zealand species of *Libertia* tested. This shows that *A. nonstriata* does not pose a direct threat to native New Zealand flora, particularly *Libertia* spp., the closest native relatives to *I. pseudacorus*, and of which some member species are endangered.

In conclusion, host range testing of *A. nonstriata* determined that the host range of *Aphthona nonstriata* is restricted to species belonging to the genus *Iris* and that the beetle is highly unlikely to pose a threat to New Zealand native flora.

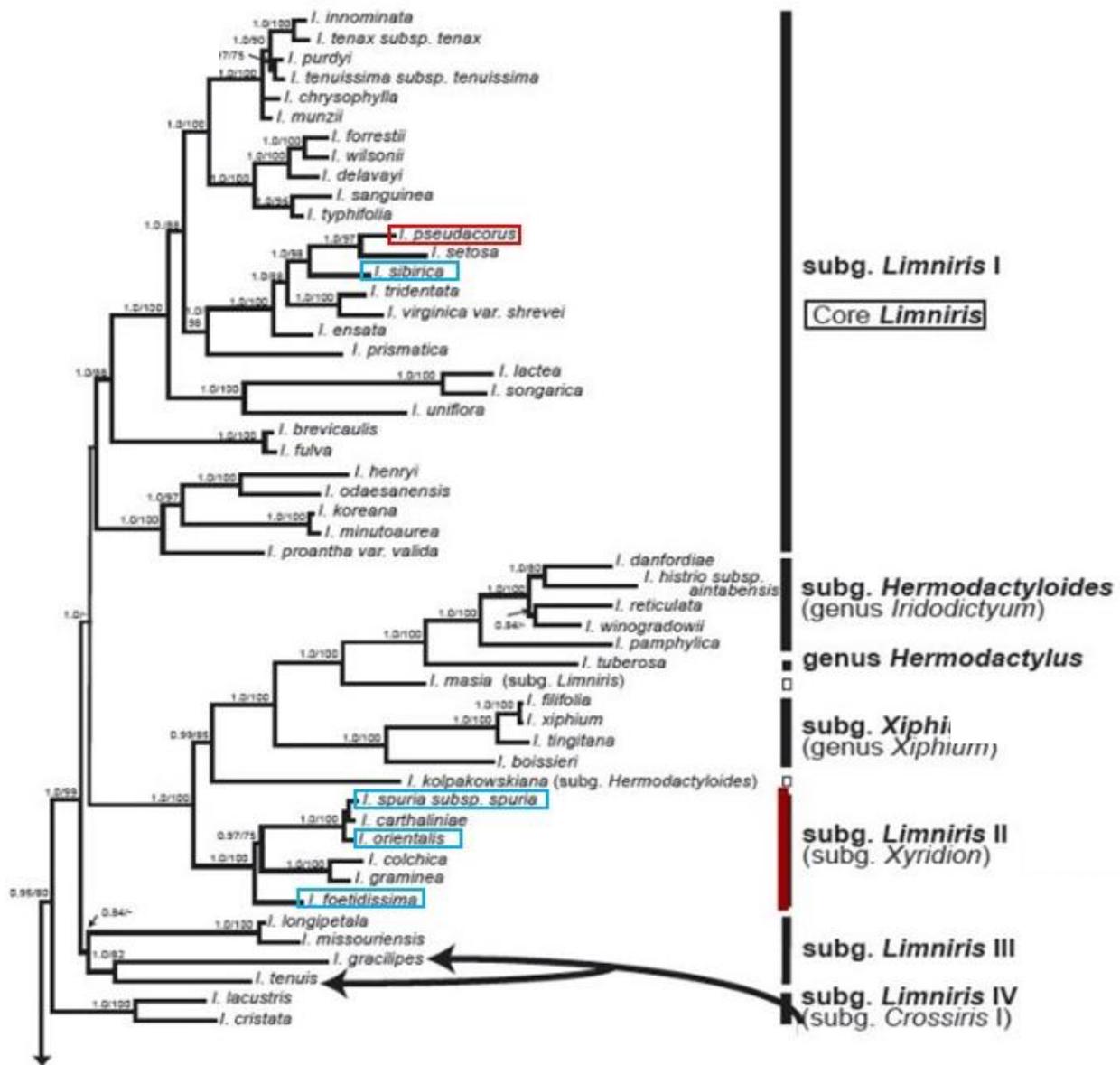


Figure 9. Phylogenetic relationships among *Iris* species. The red box indicates *I. pseudacorus* position. Blue boxes indicate positions of *Iris* species that supported *A. nonstriata* F1 development in our trials. *I. laevigata* and *I. confusa* not shown.

(Source: Adapted from Crespo 2011 with permission.)

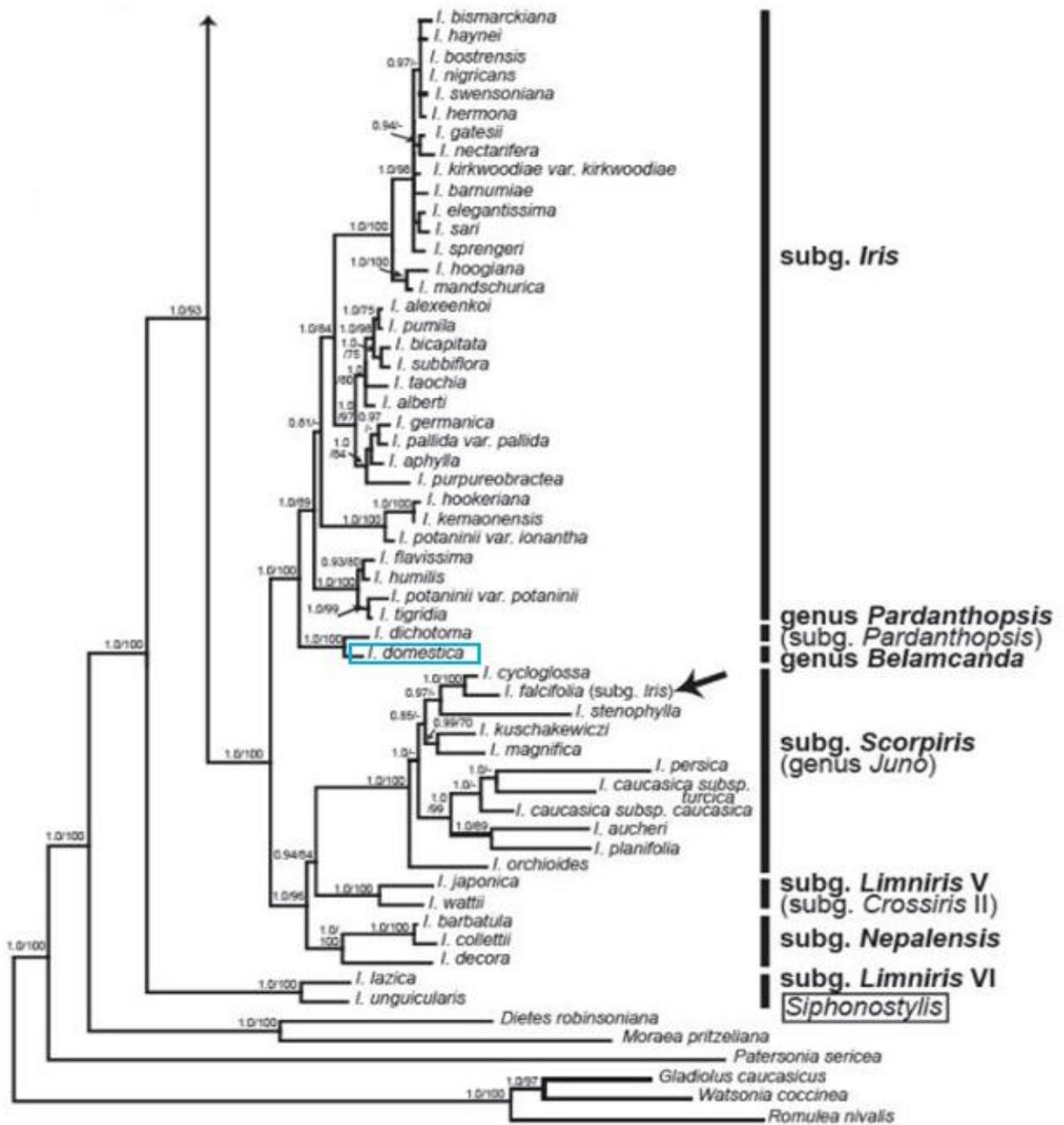


Figure 10 . Phylogenetic relationships among *Iris* species. The blue box indicates the position of *Iris* species ( $n = 1$ ) that supported *A. nonstriata* F1 development in our trials. (Source: Adapted from Crespo 2011 with permission.)

## 7 Recommendations

Based on our study, we recommend that the biological control programme targeting *I. pseudacorus* proceeds with an application to the Environmental Protection Authority to release *A. nonstriata* as a biocontrol agent for *I. pseudacorus* in New Zealand.

## 8 Acknowledgements

The *Iris pseudacorus* biocontrol programme is funded by MPI as part of the Multi-weed Biocontrol grant S3F20095, and by the National Biocontrol Collective, with additional support from the Waikato Regional Council. We thank our collaborators at the Centre for Biological Control at Rhodes University, South Africa, and Vrije Universiteit in Brussels, Belgium, for their contributions to the host range testing of *Aphthona nonstriata*.

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