Genetic diversity and morphological variation in African boxthorn (*Lycium ferocissimum*) – Characterising the target weed for biological control


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ABSTRACT

*Lycium ferocissimum* (African boxthorn) is a Weed of National Significance in Australia. Biological control may have potential to manage this weed, but taxonomic uncertainty needs to be addressed first to facilitate searches for potential agents. We sampled putative *L. ferocissimum* (i.e. tentatively identified morphologically in the field) across its native range in South Africa and introduced range in Australia. Morphometric and genetic analyses were conducted to confirm the species identity of these samples, and to assess morphological and genetic variation across both ranges. All samples collected in Australia were confirmed as *L. ferocissimum*, with no evidence of hybridisation with any other *Lycium* species. Nuclear and chloroplast genetic diversity within *L. ferocissimum* across both South Africa and Australia was low, with no evidence of genetic structure. One of the two common chloroplast haplotypes found across Australia was found at only two sites in South Africa, both near Cape Town, suggesting that the Australian lineage may have originated from this region. Ten samples from South Africa putatively identified in the field as *L. ferocissimum* were genetically characterised as different (unidentified) *Lycium* species. Our morphometric analyses across different *Lycium* species in South Africa did not identify any leaf or floral characteristics unique to *L. ferocissimum*, and thus morphological identification of the latter species in its native range may remain problematic. To ensure the correct *Lycium* species is surveyed for candidate biological control agents we suggest that individuals should be permanently tagged and putative morphological determinations supplemented with genetic analyses to confirm species identity.

1. Introduction

*Lycium ferocissimum* Miers (African boxthorn; Solanaceae) is a large spine-covered shrub native to South Africa (Arnold and De Wet, 1993; Venter, 2000). It was widely planted in Australia as a hedge plant during the mid-1800s, but has subsequently developed into an aggressive invader of pastures, roadsides and reserves across large areas of the country (Parsons and Cuthbertson, 1992). *Lycium ferocissimum* can form dense thickets that are impenetrable barriers to grazing animals. These thickets reduce the value of grazing land significantly and can harbour pests such as rabbits and foxes (Noble and Rose, 2013). The extensive environmental and economic damage caused by *L. ferocissimum* is one of the reasons this species was designated a Weed of National Significance in 2012 (Australian Weeds Committee, 2013).

Control of *L. ferocissimum* in Australia is challenging. It has a broad distribution across a range of environments and it is resilient to both chemical and mechanical management techniques (Noble and Rose, 2013). Biological control is considered a potential option to complement conventional control methods for *L. ferocissimum* in Australia (Adair, 2013; Ireland et al., 2019a). A prerequisite during the initiation of a biological control program is the clear taxonomic identification of the weed in question, in both its native and introduced ranges (Gaskin et al., 2011). Interspecific hybrids in plants are said to be particularly successful invaders (Ellstrand and Schierenbeck, 2000; Lee, 2002; Schierenbeck and Ellstrand, 2009), so it is paramount to determine if the invasive Australian *L. ferocissimum* lineage is of hybrid descent, especially considering that hybridisation among *Lycium* species is considered common in South Africa (Minne et al., 1994; Venter et al., 2000).
2003a,b). In addition, hybridisation between invasive and native species is not uncommon (Paul et al., 2010), and can significantly affect potential biological control strategies (Gaskin et al., 2011). Lycium australis F. Muell., the single Lycium species native to Australia, has an overlapping distribution with L. ferocissium across parts of Western Australia, South Australia, Victoria and New South Wales (Adair, 2013). The introduced “Goji berries” (either L. barbarum L. or L. chinense Mill.; Wetters et al., 2018), of Eurasian origin, are also grown in South Africa (Randall, 2007). Likewise, Lycium afrum L., of African origin, has been introduced and is naturalised in Australia (Randall, 2007).

Molecular tools are commonly used to complement morphological characters for the taxonomic identification of weeds (Gaskin et al., 2011). In addition, these tools can be used to assess evidence of hybridisation, and to determine the provenance of invasive weeds (Gaskin et al., 2011; Sutton et al., 2017; Williams et al., 2005). However, accurate taxonomic identification of the target weeds using morphological characters in the field remains vital, particularly when conducting comprehensive native range surveys for candidate biological control agents across broad geographic regions (Godsby et al., 2006; Witt, 2004). Species within the genus Lycium are highly plastic, so delimiting and identifying species within the genus with morphological characters can be difficult (Levin et al., 2006b; Venter, 2000). Morphological variation within L. ferocissium is substantial (Venter, 2000), perhaps influenced by the broad range of climatic and environmental conditions across its distribution in South Africa (Fig. 1). Identification of this species in the field is therefore challenging.

In this study, we collected samples from putative L. ferocissium plants (i.e. tentatively identified morphologically in the field) from across its native range in South Africa and invaded range in Australia. Genetic analyses (three chloroplast markers, one nuclear marker) were conducted in order to: (a) confirm plant identity, (b) assess genetic structuring across the native and invaded ranges (to explore the provenance of the invasive lineage), and (c) assess evidence for hybridisation between L. ferocissium and other Lycium species that occur in Australia. In addition, we undertook morphometric measurements of a number of plants from South Africa and Australia. This allowed us to quantify the extent of morphological variation within L. ferocissium, determine whether reliable morphological traits can aid in-field identifications of plants, and assess whether morphological differences exist across plants from South Africa and Australia.

2. Material and methods

2.1. Sampling

The native distribution of L. ferocissium in South Africa was collated from South African herbarium records and the South African National Biodiversity Institute online database (https://www.sanbi.org/)(Fig. 1). Plants were subsequently located during roadside surveys, and putatively identified as L. ferocissium based on fruit characteristics and flower shape (Venter, 2000). At least one plant was tagged at all sites (Fig. 1). At several sites significant morphological variation was evident, so two or more plants were tagged. Global Positioning System (GPS) co-ordinates were recorded for each tagged plant, and morphometric measurements taken (see Section 2.3). Leaf samples for genetic analyses were taken from tagged plants, and from a maximum of ten surrounding plants growing within 100 m of the tagged individual. Leaf samples were stored dry in envelopes with silica gel to preserve the DNA.

The distribution of L. ferocissium across Australia was obtained from the Atlas of Living Australia (ALA, 2016). Leaf samples were taken for genetic analysis from up to 12 individual plants per site from across the species’ distribution in Australia (Fig. 1). Morphometric measurements were taken from plants from an additional 21 haphazardly selected sites distributed along regional roads and on farms in Queensland and New South Wales in February 2019. Flowering individuals were found at only six of these sites during this survey, so data on floral traits were recorded only from these sites in Australia.

2.2. DNA extraction, amplification, sequencing, and genetic analyses

We sequenced samples identified morphologically as L. ferocissium from 41 sites across its native range (South Africa), and 43 sites across the invaded range (Australia; Fig. 1; Table S1). One to four samples were sequenced per site. DNA was initially extracted from leaf tissue using the CTAB extraction protocol (Doyle and Doyle, 1987). This was followed by a do-it-yourself spin column extraction with EconoSpin spin columns from Epoch Life Sciences (Missouri City, Texas), using the protocol of Ridley et al. (2016). Four genomic regions were amplified: three chloroplast markers (trnH-psbA intergenic spacer, matK K, and the trnT-trnL intergenic spacer), and one nuclear marker (grl larate bound starch synthase; GBSSI). The trnH-psbA intergenic spacer region was amplified using primers trnH and psbA3f (Sang et al., 1997; Tate and Simpson, 2003), for matK we used matK-1RKf-m and matK-3FKM (Kuzmina et al., 2012), for the trnT-trnL intergenic spacer we used trnL-a and trnL-b (Taberlet et al., 1991), and for GBSSI we used waxYF and waxY2R (Levin et al., 2006a; Miller et al., 1999). PCRs (12 µL) contained 7.6 µL water, 2.0 µL 5x buffer (Bioline, London, UK), 0.24 µL of each of 10 mM forward and reverse primer, 0.08 µL Taq polymerase (Bioline, London, UK) and 2 µL of DNA template. Cycling conditions for the chloroplast markers involved an initial denaturation step (95 °C for 3 min) followed by 40 cycles of 95 °C for 20 s, 50 °C for 45 s, and 72 °C for 45 s, with a final annealing step of 72 °C for 10 min. A touchdown protocol was used to amplify GBSSI, following the protocols of Levin and Miller (2005). PCR amplification was confirmed by gel electrophoresis, with amplified DNA cleaned with 1 µL of Exonuclease I (New England Biolabs Inc, Ipswich, United States) per sample. Samples were sequenced in both directions by Macrogen Inc. (Korea).

Sequence chromatograms were edited in Geneious v11.1.5 (https://www.geneious.com; Kearse et al., 2012), and aligned with additional sequences of South African and Asian Lycium species, and two out-groups (Capsicum lanceohydris Bitter and Solanum tuberosum L.) from GenBank (Table S2), using the MUSCLE plugin (Edgar, 2004). We used JModeltest v2.1.2 (Darriba et al., 2012) to select the best model of sequence evolution for each marker. The three chloroplast regions were concatenated into a single dataset, as each exhibited low levels of sequence divergence, and there should be no incongruence among regions of the plastid genome as they cannot assort independently or recombine. Phylogenetic relationships were reconstructed in MrBayes 3.2.6 (Huelsenbeck and Ronquist, 2001). The concatenated chloroplast dataset was partitioned by DNA region, allowing independent estimation of parameters for each partition. Indels were treated as missing data. Four Markov chains were run for five million generations, with chains sampled every 200 generations. The first 2,000 trees were discarded as burn-in. We used Tracer v1.7.0 (Rambaut et al., 2018) to confirm that all parameters had converged, that burn-in was sufficient, and that the effective sample size was greater than 200 for each prior.

2.3. Morphometric analyses

The following morphometric measurements were recorded for each of 48 plants from South Africa, including more than one plant per site in some instances, and 21 plants from different sites in Australia (Tables S3, S4): Plant. Plant height (measured as the highest leaves on the plant in relation to the ground), plant width (measured at its widest part from...
branch tip to branch tip), and **stem diameter** (recorded at ground level).

**Leaves.** Ten of the largest leaves from ten haphazardly selected branches were excised, and we measured **leaf length** (measured from lamina tip to the petiole along the lamina midrib), and **leaf width** (measured at the widest part of the leaf). In addition, we removed a branch from each plant (which was deposited in the herbarium; as detailed below) and counted the **number of leaves per fascicle**.

**Flowers.** A mature flower, where present, was haphazardly excised from each plant and dissected to show a transverse section of the flower. A photograph of the section with scale bars was then taken, and this was used to determine **flower shape** (tubular or bell), and **sexuality**, and to measure **flower dimensions** (Fig. 2). These floral characters have previously been used to differentiate between distinct *Lycium* species (Venter, 2000).

Herbarium specimens were taken from all plants in South Africa for which morphometric characters were recorded in the field. Specimens were examined in the Selmar Schonland Herbarium, Grahamstown, South Africa to verify in-field identifications using a dichotomous key to African *Lycium* species (Venter, 2000), and voucher specimens lodged (Tables S3, S4). The defining characters for *L. ferocissimum* were shiny bright green obovate to elliptic leaves, 12–35 mm long, 4–10 mm wide and a flower calyx 2/3 the length of the corolla tube (Venter 2000). Representative voucher specimens of Australian *L. ferocissimum*...
plants were deposited in the Australian National Herbarium, Canberra (accession codes: CANB912039 and CANB912040; Tables S3, S4). Based on DNA sequencing results, morphometric measurements made in South Africa were allocated to two groups: *Lycium ferocissimum* and "*Lycium* sp. unknown". A third group consisted of measurements made on *Lycium ferocissimum* in Australia. Statistical differences in plant morphology among groups were assessed using STATISTICA 10 (Hill and Lewicki, 2007). Analysis of Variance (ANOVA) were conducted for each of the plant characters measured. Where significant differences were detected, Fisher’s Least Significant Difference (LSD) tests were used to make post hoc comparisons across groups. Differences in flower shape and sexuality were assessed using Chi-Square statistics.

3. Results

3.1. Genetic analyses

We sequenced three chloroplast markers from 217 plants across 84 sites – 89 plants from 41 sites in South Africa and 128 plants from 43 sites in Australia (GenBank accession numbers: MN910317–MN910965). Following concatenation of these distinct markers, the edited DNA alignment was 2,152 bp in length. Limited genetic diversity was detected in the concatenated chloroplast dataset, with only eight haplotypes identified across the material from South Africa and Australia. All plants at most sites were characterised by only a single haplotype, though at some sites two haplotypes were recovered (see below).

Plants tentatively identified with morphological characters in the field as *L. ferocissimum* did not form a monophyletic clade in the Bayesian phylogeny (Fig. 3). Most of the South African samples, and many of the Australian samples, were identical across the three chloroplast markers, and matched sequences of *L. ferocissimum* accessions from GenBank (haplotypes SA common and AU common 1; Fig. 3; see Table S5 for a list of sites where this haplotype was found). Samples from several locations in the Eastern (EC15, EC19) and Western Cape (WC10, WC11, WC13, WC14, WC16; see Fig. 1) Provinces of South Africa did not match any *Lycium* accessions on GenBank but were included in the well-supported clade with *L. ferocissimum* (clade A; Fig. 3). Samples from two of these sites (WC14, WC16) shared a haplotype that was also common in Australian samples (haplotype AU common 2; Fig. 3; see Table S5 for a list of sites where this haplotype was found). *Lycium ferocissimum* samples in clade A were not monophyletic, with Clade A also including three other *Lycium* species (Fig. 3). For the purposes of the morphometric analyses (Section 3.2) all of the samples from Clade A were considered to be *L. ferocissimum*.

Plants from eight sites in South Africa, putatively identified morphologically as *L. ferocissimum*, were not included in clade A with the remaining *L. ferocissimum* samples. These putative *L. ferocissimum* sequences did not match any sequences in GenBank, and their phylogenetic position suggests that they are not *L. ferocissimum*. These samples were part of an unresolved polytomy containing 12 other *Lycium* species (Fig. 3). These plants were classified as "*Lycium* sp. unknown" and placed in a separate group for the morphometric analyses (see Section 3.2). A sample from site WC1 had a unique chloroplast haplotype, but the phylogenetic placement of this sample was not well resolved (Fig. 3). Only a single *Lycium* species was recorded at most of our collection sites across South Africa (Table S1). However, at three sites (EC2, EC3, WC1) both *L. ferocissimum* and "*Lycium* sp. unknown" were recorded (Table S1).

We sequenced the GBSSI gene from 199 plants across 84 sites – 79 plants from 41 sites in South Africa and 120 plants from 43 sites in Australia (GenBank accession numbers: MN910966–MN911163). Eighteen discrete GBSSI haplotypes were identified (Fig. 4). Only one of these haplotypes was common across Australia and South Africa (labelled SA common and AU common; see Table S5 for a list of sites where this haplotype was found). The remaining haplotypes were typically restricted to only a single site (Fig. 4). A single GBSSI haplotype was found at most sites, though two haplotypes were recorded at some sites (e.g. EC3; Fig. 4). The relationships among the GBSSI haplotypes were not well resolved (Fig. 4), and the GBSSI and chloroplast phylogenies were not completely congruent. However, all of the specimens from clade A in the chloroplast phylogeny (considered to be *L. ferocissimum*) were included in a single clade in the GBSSI phylogeny (which also includes the *L. ferocissimum* accession from GenBank). This...
clade, however, was only weakly supported (PP = 0.70). The clade also includes *L. afrum* and *L. schizocalyx* (as it did in the chloroplast phylogeny), but not *L. tenue* (Fig. 4). All samples classified as “*Lycium* sp. unknown” in the chloroplast phylogeny were again genetically distinct from those confirmed as *L. ferocissimum* (Fig. 4). Most of these samples were included in a clade in the GBSSI phylogeny with *L. tenue*, *L. gariepense*, and *L. australis*, though this clade was poorly supported (PP = 0.60). The sample from site EC9, however, was most closely related to *L. oxycarpum* (Fig. 4).

No evidence of a genetic disjunction within *L. ferocissimum* across the Western and Eastern Cape Provinces of South Africa was apparent, with a single common chloroplast and GBSSI haplotype shared across the two regions. There was no obvious conflict between the placement of our *L. ferocissimum* samples in the GBSSI and chloroplast phylogenies...
Table 1
Comparisons of morphological characteristics across plants of *Lycium ferocissimum* from South Africa (n = 37) and Australia (n = 21), and “*Lycium sp. unknown*” (n = 10) examined in South Africa (Tables S3, S4). The identification of *L. ferocissimum* and “*Lycium sp. unknown*” in South Africa is based on results from the genetic analysis. Results of ANOVAs and χ² tests across the three groups are also displayed, with superscript letters denoting a statistically significant difference across groups at p < 0.05.

<table>
<thead>
<tr>
<th>Lycium ferocissimum</th>
<th>“Lycium sp. unknown”</th>
<th>ANOVAs and χ² tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Africa</td>
<td>Australia¹</td>
<td></td>
</tr>
<tr>
<td>Sexual dimorphism</td>
<td>Bisexual (34), asexual (3)ᵇ</td>
<td>Bisexual (6)ᵇ</td>
</tr>
<tr>
<td>Flower shape (no. of sites)</td>
<td>Bell (29), tubular (8)b</td>
<td>Bell (6)b</td>
</tr>
<tr>
<td>Length AC (±1SE) (mm)</td>
<td>17.0 (± 3.9)ᵇ</td>
<td>11.8 (± 2.3)ᵇ</td>
</tr>
<tr>
<td>Width BD (±1SE) (mm)</td>
<td>9.4 (± 2.0)ᵇ</td>
<td>8.8 (± 1.0)ᵇ</td>
</tr>
<tr>
<td>Length AB (±1SE) (mm)</td>
<td>12.0 (± 3.0)ᵇ</td>
<td>7.4 (± 2.0)ᵇ</td>
</tr>
<tr>
<td>Length BC (±1SE) (mm)</td>
<td>8.0 (± 2.4)ᵇ</td>
<td>7.4 (± 2.0)ᵇ</td>
</tr>
<tr>
<td>Stem characteristics</td>
<td>Diameter (±1SE) (mm)</td>
<td>63.9 (± 46.0)ᵇ</td>
</tr>
<tr>
<td>Plant characteristics</td>
<td>Height (±1SE) (m)</td>
<td>1.80 (± 0.80)</td>
</tr>
<tr>
<td>Width (±1SE) (m)</td>
<td>2.5 (± 1.1)</td>
<td>2.4 (± 1.0)</td>
</tr>
<tr>
<td>Leaf characteristics</td>
<td>Leaves per fascicle</td>
<td>7.5 (± 2.1)</td>
</tr>
<tr>
<td>Length (±1SE) (mm)</td>
<td>28.1 (± 8.6)b</td>
<td>21.0 (± 5.3)b</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>8.1 (± 3.8)</td>
<td>8.6 (± 2.2)</td>
</tr>
</tbody>
</table>

¹ Floral characteristics were assessed for only six individuals plants, because of lack of flowering at the time to survey was conducted.

(Figs. 3, 4), providing no evidence for hybridisation in the native range. Likewise, no evidence of hybridisation was apparent in Australian plants, with all plants from Australia having only *L. ferocissimum* chloroplast and nuclear DNA.

### 3.2. Morphometric analyses

Both asexual and bisexual, and bell-shaped and tubular flowers were recorded from South African plants molecularly identified as *L. ferocissimum*, though bisexual and bell-shaped flowers were the most common (Table 1; Table S3). In contrast, most of the “*Lycium sp. unknown*” plants had asexual and tubular flowers, with Chi-squared tests revealing significant differences in these characteristics across *L. ferocissimum* and “*Lycium sp. unknown*” (Table 1). There were, however, no significant differences across these two groups in any other morphological trait measured (Table 1).

Only bisexual bell-shaped *L. ferocissimum* flowers were recorded in Australia (Table 1). Differences in a number of floral characteristics were detected across *L. ferocissimum* plants from South Africa and Australia (Table 1). Most notably, plants from Australia had a significantly wider corolla’s, but significantly shorter AC (length from the top of the stalk to the corolla petals) and AB (length from the top of the stalk to the highest point of corolla) measurements (see Fig. 2). In addition, South African *L. ferocissimum* plants had significantly larger diameter stems and longer leaves than those of Australian plants (Table 1; Table S4).

### 4. Discussion

#### 4.1. Genetic and morphological variation across Lycium samples collected in South Africa

Our study highlights the challenges that must be dealt with when searching for candidate biological control agents for *L. ferocissimum* across its native range. While most specimens collected across South Africa that were morphologically identified as *L. ferocissimum* were confirmed as that species, genetic analyses revealed that several specimens (particularly from the Eastern Cape Province) were not included in the clade that included *L. ferocissimum*. It is possible that these plants are indeed *L. ferocissimum*, with the discrete chloroplast lineages resulting from incomplete lineage sorting. However, these samples group together and are genetically distinct from samples confirmed as *L. ferocissimum* in both the nuclear and chloroplast phylogenies, even at sites where the discrete chloroplast lineages occur in sympatry (EC2, EC3, WC1). This suggests an absence of gene-flow across lineages, indicating that these samples are most likely from different *Lycium* species. Identifying these non-*L. ferocissimum* plants to species level was not possible based on sequence data currently available in GenBank. Nonetheless, the genetic analysis suggests that more than a single unidentified *Lycium* species was included in our sampling. Several of these plants may represent interspecific hybrids between *L. ferocissimum* and one or more other *Lycium* species, though further well-designed tests of this conjecture are required. Indeed, these unidentified species could potentially represent undescribed *Lycium* species. South Africa is considered one of the centers of diversity for the genus *Lycium* (Venter, 2000), but few studies have been conducted on the genus across the country. A more thorough investigation of the genus across South Africa – using a combination of alpha taxonomy and DNA sequencing – is clearly required.

Confirmation that several of the plants putatively identified as *L. ferocissimum* in the field using morphological characters were from different (unidentified) *Lycium* species is cause for concern in the context of surveying for candidate biological control agents. Whereas floral traits, such as shape and sexuality, may assist to distinguish *L. ferocissimum* from other *Lycium* species, they may still not do so with complete accuracy. Furthermore, flowers are not always present, and our analyses highlighted that identification based on other morphological characters is not reliable. To overcome this problem, we have been surveying for potential biological control agents on permanently tagged individual *L. ferocissimum* plants for which the identity has been confirmed by DNA sequencing. Further, we have deemed it prudent to keep specimens and colonies of candidate biological control agents collected from different plants separate, until their identity as *L. ferocissimum* is confirmed.

#### 4.2. Genetic and morphological variation across L. ferocissimum from South Africa and Australia

The identification of *L. ferocissimum* in the field in Australia was not as problematic as in South Africa. This is because there are only four
other *Lycium* species that are present, outside of cultivation, but with restricted distributions: the native *L. australe* and the naturalised *L. barbarum, L. chinense*, and *L. arium* (Randall, 2007). Our genetic analyses confirmed that all samples morphologically identified as *L. ferocissimum* were indeed that species. No evidence of hybridisation between any of the *Lycium* species in Australia was detected in the analyses. This is encouraging because invasive weeds that are hybrids are typically considered to be more challenging to target with biological control (Williams et al., 2014; Zalucki et al., 2007).

Few morphological differences were detected in *L. ferocissimum* leaf or stem characteristics across South Africa and Australia. However, significant differences in flower structure were evident among plants from South Africa and Australia. These differences could: (a) indicate that only plants with genetic material for shorter, bell-shaped flowers were introduced into Australia; (b) be the result of phenotypic plasticity related to environmental conditions (see Gratani, 2014), or (c) suggest that the reduced selection pressure in Australia (e.g. due to fewer herbivores; see Adair, 2013) resulted in plants investing in reproduction and growth rather than herbivore defenses (see Blossey and Notzold, 1995), leading to larger flowers. These morphological differences in flower shape and size are, however, unlikely to affect future biological control agents, unless flower-feeding agents are released.

Genetic diversity across *L. ferocissimum* in South Africa was low based on the markers used in this study, and even less genetic diversity was found in *L. ferocissimum* in Australia, a phenomenon commonly observed in invasive plants (Ward et al., 2008). The lack of any detected genetic diversity and structure across *L. ferocissimum* in South Africa makes inferring the introduction history of the invasive lineage challenging. However, one of the two chloroplast haplotypes found in Australia was identified in South African material only from plants collected in the Western Cape Province, near Cape Town. The other common chloroplast haplotype identified in Australia was also found in plants from this area, as well as other regions in South Africa. This suggests that the region around Cape Town may be the provenance of the invasive Australian lineage, though the possibility that *L. ferocissimum* was introduced to Australia from multiple localities cannot be excluded.

Local adaptation is widely believed to lead natural enemies to be more damaging to plants from populations with which they have co-evolved locally (Kniskern and Rausher, 2001). Results of a recent investigation into the rust fungus *Puccinia rapipes* Berndt & Ulmann, a candidate biological control agent found on *L. ferocissimum* in South Africa, are consistent with this conjecture. In this study an isolate of this rust fungus from the Western Cape was significantly more pathogenic on *L. ferocissimum* plants from Australia, than an isolate from the Eastern Cape (Ireland et al., 2019b). Future studies should employ next-generation-sequencing approaches, such as genotyping-by-sequencing (see Burrell et al., 2015), to reconstruct the invasion history of *L. ferocissimum* in Australia more accurately to test this proposition further.

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CRediT authorship contribution statement

G.A. McCulloch: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. E.V. Mauda: Formal analysis, Investigation, Writing - review & editing. L.D. Charì: Formal analysis, Investigation, Writing - review & editing. G.D. Martin: Formal analysis, Investigation, Writing - review & editing. K. Gurdasani: Investigation, Writing - review & editing. L. Morini: Conceptualization, Investigation, Funding acquisition, Project administration, Writing - review & editing. G.H. Walter: Conceptualization, Writing - review & editing. S. Raghu: Conceptualization, Investigation, Funding acquisition, Project administration, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocntrol.2020.104206.

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