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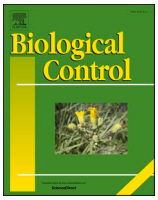

Risk assessment of the laboratory host range and a molecular characterisation determining the field host range of *Lixus aemulus,* for the biological control of *Chromolaena odorata* in South Africa

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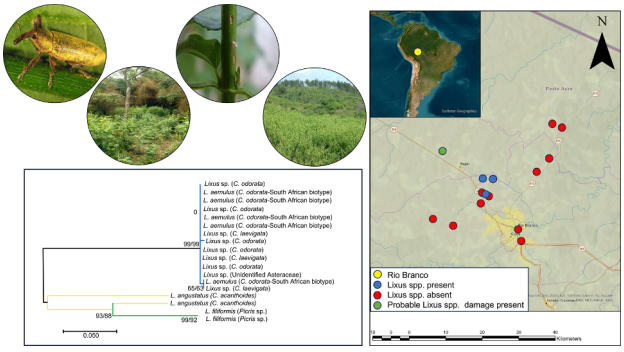
HIGHLIGHTS

- Laboratory tests revealed that *Lixus aemulus* is host-specific to *Chromolaena odorata.*
- *L. aemulus* may utilise closely related invasive plants but not indigenous, ornamental or crop species in South Africa.
- Native range exploration confirms *L. aemulus* present on *C. odorata.*
- Genetic assessments show *Lixus* sp.(p.). adults collected on various hosts, limited to the Eupatorieae tribe, as *L. aemulus*.
- Limited establishment of *L. aemulus* in South Africa may be attributed to a climatic mismatch with Brazilian collection sites.

GRAPHICAL ABSTRACT



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ABSTRACT

Chromolaena odorata (Asteraceae: Eupatorieae) is a sprawling shrub native to the Americas, and a destructive invader of much of the humid tropics and subtropics of the Old World. Opportunistic native-range exploration in 1995 identified a stem-boring weevil, *Lixus aemulus*, as a promising biological control candidate agent. Host-specificity testing was conducted on *L. aemulus* in South Africa using laboratory no-choice and paired-choice tests. Three invasive alien plants closely related to *C. odorata* may be utilized by *L. aemulus* but no indigenous, ornamental or crop species in South Africa was or is expected to be attacked by the weevil. A native-range field survey was conducted in Brazil to determine the exact identity of the host plant *L. aemulus* had been

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collected in 1995, and to identify additional host-plant species. Genetic assessments of the *Lixus* sp.(p.). adults collected on the three host plants (*C. odorata*, *Chromolaena laevigata* and *Heterocondylus vitalbae*) reveal these individuals are *L. aemulus* and the weevil can be classed as an oligophage in its native range. Over 5,500 adults were released in South Africa, but overall establishment has been poor. The most likely explanation appears to be a climate mismatch between the region of South Africa invaded by *C. odorata* and the collection locality in Rio Branco, Acre state, Brazil. Additionally, because the full extent of the native range of *L. aemulus* is unknown, it is uncertain whether individuals can be sourced from an area whose climate resembles that of South Africa. Furthermore, despite being oligophagous, *L. aemulus* may perform sub-optimally on the southern African *C. odorata* biotype.

1. Introduction

Chromolaena odorata (L.) R.M.King & H.Rob. is a perennial shrub species belonging to the tribe Eupatorieae of the Asteraceae family (King and Robinson, 1987). The shrub is widely distributed in tropical America and has since been introduced to Asia and Africa (Cock and Holloway, 1982; Muniappan et al., 2005). This species harms biodiversity, ecotourism, agriculture and forestry, which justifies its consideration as one the worst invasive plant species in the subtropical regions of South Africa (Macdonald and Jarman, 1985; Zachariades et al., 2013). Classical biological control (CBC) of *C. odorata* in South Africa was initiated in 1988 and surveys have been undertaken in numerous Central, South and North American countries (Strathie and Zachariades, 2002). In 1997, a strategic plan was drawn up, advocating the release of a suite of damaging, host-specific insects attacking different parts of the plant, namely the leaves, stems and roots (Kluge et al., 1997). A stem borer is considered a highly desirable element in the suite for *C. odorata* because the weed has photosynthetically active stems that can produce new leaf material after being defoliated (Zachariades et al., 2002). A small culture of the stem-boring weevil, *Lixus aemulus* Petri, 1928 (Coleoptera: Curculionidae) was collected opportunistically in 1995 from a pubescent 'hairy chromolaena' in Rio Branco, Acre state, Brazil by Dr S. Neser (Kluge and Zachariades, 2006). The weevil was imported into quarantine in South Africa in late 1995 under the Department of Agriculture: Directorate of Plant Health and Quality Permit 14/2/2/1 (9/94/124). *Lixus aemulus* bred easily on the southern African biotype of the plant (Zachariades et al., 1998; Zachariades et al., 2011), which originates from the northern Caribbean region and differs substantially from *C. odorata* plants from South America (Paterson and Zachariades, 2013; Shao et al., 2018).

Identifying an agent's potential during the early stages of screening is paramount to the success of a biocontrol programme. Failure to do so can be costly in terms of resources and time, and contributes to the perception that biocontrol is a hit-or-miss strategy with risks of ecological side effects (Hoelmer and Kirk, 2005; McClay and Balciunas, 2005). Curculionids maintain a good record as biocontrol agents on weeds, with two other *Lixus* species having been successfully used against invasive alien plants in Australia: *L. cardui* Olivier, 1807 on *Onopordum* spp. (Asteraceae), and *L. cribricollis* Boheman, 1835 on *Emex australis* Steinh. (Polygonaceae) (Julien and Griffiths, 1999). Other *Lixus* species have been investigated as biocontrol agents (Schmidl, 1981; Sobhian et al., 2003). Under laboratory conditions, *L. aemulus* larvae caused significant damage to the stems of *C. odorata*, resulting in a 94 % reduction in seed output (Kluge and Zachariades, 2006).

Host-specificity testing is another crucial step in the process of introducing natural enemies for classical biological control efforts, providing the basic information upon which the safety of a proposed biocontrol agent can be assessed (Heard, 2002; McClay and Balciunas, 2005). Any risks *L. aemulus* may pose must be identified and fully assessed prior to release. Characteristically, the host-range experiments examine aspects of host selection and how the agent utilizes the target plant and non-target plants, as indicated by oviposition, adult feeding, larval feeding, larval development, adult longevity and fecundity (Heard and Van Klinken, 1998). The range of non-target plants attacked is often broader in laboratory tests than in the field (Louda et al., 2003; Mayhew, 1998; van Klinken, 2000). Laboratory host-range trials presented in this paper indicate that *L. aemulus* is quite specific but is not monophagous, as is true of most *Lixus* species (Volovnik, 2024). Additionally, the exact identity of the host plant from which *L. aemulus* had been collected was uncertain (Kluge and Zachariades, 2006).

The identification of host-specific species with potential as weed biological control agents relies on studying the host range of insect herbivores from the native range of the target weed. This encompasses the diversity of host plants these insects utilize for feeding and reproduction within their native geographical location (Goolsby et al., 2006; McFadyen, 1998; Sheppard et al., 2003). Native range exploration is considered a technically difficult and time-consuming element of biological control programmes but is the foundation upon which the research ultimately depends (Goolsby et al., 2006). Given that the identity of the original host plant remained uncertain and laboratory trials may have overestimated the realized host range, field host-range surveys conducted in 2015 in the Rio Branco area were desirable. These surveys aimed to accurately determine the host plant's identity and assess whether other species within the Asteraceae are utilized by the herbivore, thus offering a more realistic estimate of the herbivore's host range under natural conditions (Sheppard et al., 2006).

This work examines the laboratory host specificity of *L. aemulus* with no-choice and paired-choice tests, to determine if and which non-target species could potentially sustain *L. aemulus* populations in the field in South Africa. We used a DNA barcoding approach to determine whether other Asteraceae plant species share *L. aemulus*, focusing on 14 sites sampled in and around Rio Branco, Acre state, Brazil. Mitochondrial DNA (mtDNA) markers were used to establish if individuals collected on the three host plants; *C. odorata*, *Chromolaena laevigata* (Lam.) R.M.King & H.Rob. and *Heterocondylus vitalbae* (DC.) R.M.King & H.Rob., together with representatives of the South African laboratory culture, are conspecific. In addition, the notion of a climatic mismatch was explored by performing a climatic comparison between the native collection site of *L. aemulus* in Brazil and Sub-Saharan Africa, particularly the release sites of the weevil in South Africa.

2. Materials & methods

2.1. Biology and taxonomy of Lixus aemulus Petri, 1928

Following its collection and importation into South Africa, the beetle was identified by Dr C.W. O'Brien of Florida A&M University, Tallahassee in 1997, as "*Lixus aemulus* Petri (Coleoptera: Curculionidae) (or near)" (Zachariades, 2004). *Lixus aemulus* was originally described from a single female collected in an unspecified region of Brazil (Petri, 1928). A similar species was collected on *C. odorata* in Venezuela in 1999 (Zachariades et al., 2011). It lacked the pink cuticular wax that is present on the head of *L. aemulus* (Fig. 1). The weevil, collected at three locations across a 180 km distance in north-western Venezuela, was identified by C.W. O'Brien in 2020 as *Lixus* sp. (all belonging to a single species), distinct from *L. aemulus*. This illustrates the potential diversity of *Lixus* species on *C. odorata* across the South American continent.

Life-history metrics for *L. aemulus* were recorded in the quarantine

glasshouse and laboratory facility of the Agricultural Research Council, Plant Health and Protection (ARC-PHP), Cedara, KwaZulu-Natal province, South Africa, at 23–31 °C and relative humidity of 45–80 %. In the laboratory, *L. aemulus* adults feed on young *C. odorata* leaves, causing minor damage (Zachariades et al., 2002). After a preoviposition period of about one month, the female inserts single eggs into green (non-woody) *C. odorata* stems containing pith (Fig. 1). The egg hatches after about one week and the larva feeds inside the green stem before pupating inside a chamber within the stem. Adult progeny cut a circular or oval hole through the stem wall before emerging. In the laboratory, adults emerge *en masse* in the spring. Adults typically live for over six months (n = 21 for which this was recorded), and the period from oviposition to adult emergence from the stem is 3–4 months, although where oviposition occurs later in the summer, overwintering occurs within the stem over winter. In the laboratory, therefore, there are 1–2 generations per year.

2.2. Selection of test plants

The test plant list for *L. aemulus* (Supplementary Table S1) was compiled using (i) standard centrifugal testing principles (Wapshere, 1974), (ii) host records for other *Lixus* species (Zachariades et al., 1999) (iii) stem morphology and (iv) taxa on which other species of *Lixus* have been recorded as agricultural pests. A test list was initially published by Zachariades et al. (2002). The asteraceous tribe Eupatorieae, and closely related tribes such as Heliantheae, which form a composite termed "the Heliantheae Alliance" (Baldwin, 2009), are poorly represented in the paleotropics. Furthermore, there are no native South African genera in the same subtribe (Praxelinae) as *C. odorata*. There are only three native genera of Eupatorieae in South Africa, viz. *Adenostemma* (subtribe Adenostemmatinae – two species), *Mikania* (Mikaniinae – two species) and *Stomatanthes* (subtribe Eupatoriinae – one species) (Retief, 2002). Only one species of *Adenostemma* was included in the host-range list because *A. viscosum* J.R.Forst. & G.Forst. has stems that are morphologically similar to those of *A. caffrum* DC. – both are succulent with no pith and high water content, such that the *L. aemulus* larvae cannot survive. *Mikania natalensis* DC. was not tested as it was not found in the field; it is very similar to *M. capensis* DC, and may not be a separate species (W.C. Holmes, Baylor University, 2013, pers. comm. to C. Zachariades). The herbaceous perennial *Stomatanthes africanus* (Oliv. & Hiern) R.M.King & H.Rob., the only native species to have been a former congener of *C. odorata* (both *Eupatorium*), grows in high-altitude grasslands, and has extremely thin stems. Several less closely related Asteraceae were excluded if their stem morphology was judged unsuitable for larval survival of *L. aemulus* (e.g. no pith, high water content, thin and herbaceous). Conversely, several non-asteraceous crop plants (sweet potato, *Ipomoea batatas* L. (Convolvulaceae), tomato, *Solanum lycopersicum* L. (Solanaceae) and squash and pumpkin, *Cucurbita pepo* L. (Cucurbitaceae)) were included because they were judged to have physically suitable stems for *L. aemulus* development. Finally, *Lixus brevirostris* Boheman, *L. incanescens* Boheman, *L. juncii* Boheman and *L. scabricollis* Boheman are known pests of beet (*Beta vulgaris* L. (Amaranthaceae)); *Hypolixus haerans* (Boheman) and *H. truncatulus* (F.), both formerly in *Lixus*, of amaranth (*Amaranthus* spp. (Amaranthaceae)); and *L. algirus* L. of faba bean (*Vicia faba* L. (Leguminosae)). Therefore these plants, or plants closely related to them, were used in host-range trials.

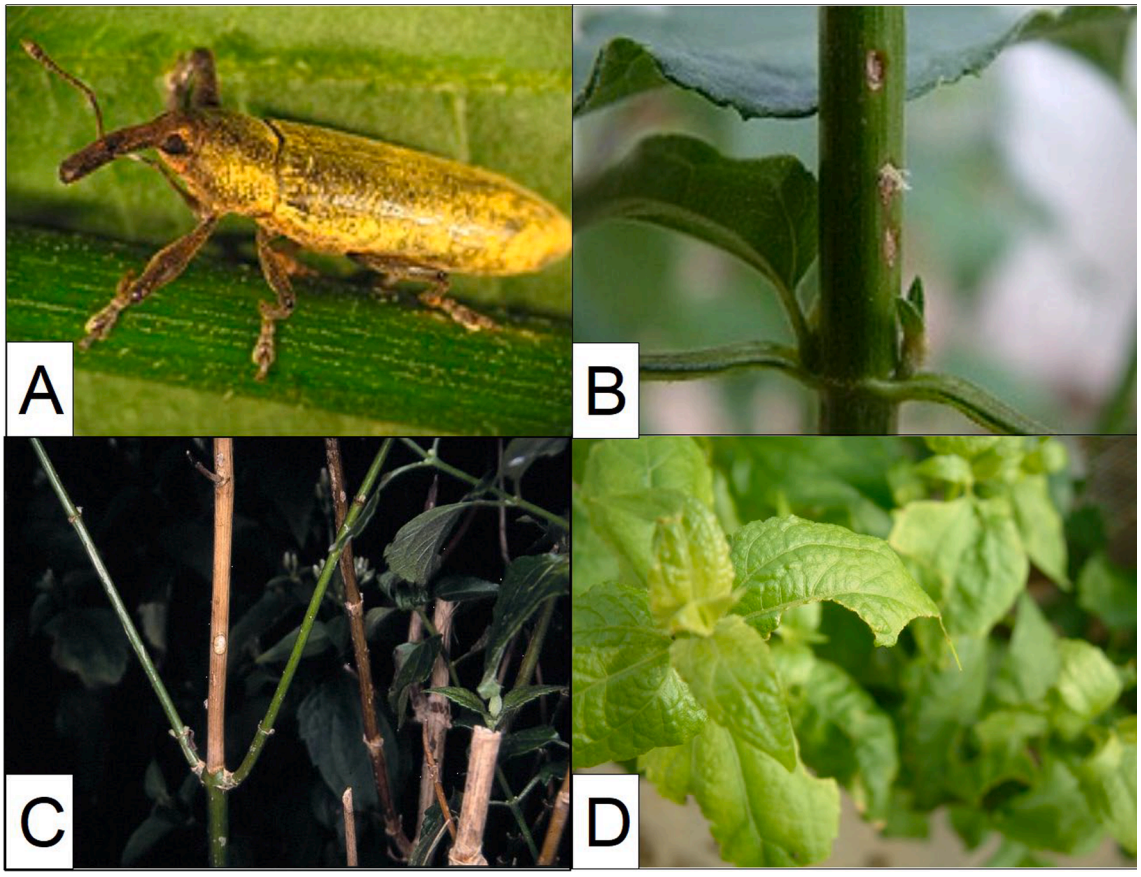

Fig. 1. A: Adult *L. aemulus*; B: *L. aemulus* oviposition holes in the stem of *C. odorata.* The female drills an inspection hole with her rostrum and if the stem is suitable inserts the egg into the stem and plugs the hole; C: Stem with *L. aemulus* larval damage and exit hole chewed by adult progeny; D: Adult *L. aemulus* feeding damage.

2.3. No-choice adult trials

All laboratory culturing and experimental studies were conducted in the quarantine glasshouse and laboratory facility of the ARC-PHP, Cedara, at 23–31 °C and relative humidity of 45–80 %. Host-range trials for *L. aemulus* were conducted by comparing adult mortality, feeding damage, oviposition, and larval development on *C. odorata* to that of 29 non-target plant species (Supplementary Table S1). Four mating adult pairs between 1 and 2 months old (peak egg-laying age for females) were placed into a cage (30 × 40 × 70 cm) containing a single plant. Males were distinguished from females by their shorter, thicker rostrum. Adults were provided with sprayed water once a day. Plants were replaced every 10 days, and the trial was terminated after 30 days. Each time plants were replaced, a record was made of the number of live adults remaining, but dead and missing adults were not replaced. Once plants had been removed from the cage, they were examined for adult feeding and oviposition. Leaves on which adults had fed were removed from the plant and categorised according to the percentage of leaf feeding These feeding percentages were converted to a total leaf area removed by first obtaining an average area (n = 50) for a leaf of each plant species, and then by multiplying the total percentage leaf area eaten on each plant by the average species leaf area. Oviposition holes in the stem were marked with paper tags and judged to be "plugged" i.e. containing an egg, or "unplugged" i.e. exploratory boring only. Plants on which oviposition holes were present were dissected after a period equivalent to the development time on *C. odorata*. The numbers of live and dead larvae, pupae and adults, and the number of eggs and adult emergence holes, were recorded from dissected plants. Over the estimated larval development period, some experimental plants died. These were dissected, and total larval counts were made. Trials were arranged in a series of "runs" containing four to nine non-target species and one *C. odorata* plant to control for temporal variation in *L. aemulus* behaviour. All *C. odorata* were inoculated with *L. aemulus* adults to provide a positive control. Replicates of a given non-target species were always assigned to different runs.

2.4. Adult paired-choice trials

Paired-choice trials were conducted for the non-target species that supported adult development from progeny production in no-choice adult trials. One *C. odorata* plant and one non-target plant were placed in a cage (50 × 50 × 90 cm). Four pairs of *L. aemulus* adults were introduced onto a platform between the plants. On days 1, 2, 3, 5 and 10 after introduction, the position of the adults (on control plants, test plants or elsewhere) was recorded. The plants were again replaced every 10 days, and after 30 days the trial was terminated. The same procedure was followed, and the same variables were recorded as for the no-choice trials. In addition, each time plants were replaced, their position in the cages was altered by 90° to control for directional movement of adults, adults were placed back onto the platform between the plants, and adult positions were again recorded on the same days after introduction as above.

2.5. Risk assessment

For those non-target plant species that demonstrated susceptibility to *L. aemulus,* the potential risks (feeding and reproduction) to non-target species quantified (Wan and Harris, 1997) by measuring adult feeding and survival, together with the production of progeny, on each non-target test plant species, as a proportion of that on *C. odorata*. The following performance procedures were used to assess the risk:

1) Feeding risk percentage was calculated as a product of plant preference (relative feeding damage) of the adults during paired-choice tests, multiplied by feeding damage by the adults (relative damage) during no-choice tests (Mangan and Baars, 2023).

$$\textbf{Feeding Risk} = \textbf{Plant preference} \times \textbf{Feeding damage}$$

where:

- **Plant preference** is the attractiveness of test plant species, determined by adult preference (relative feeding) in paired-choice trials.

$$\text{Relative feeding} = \frac{\text{Feeding on non} - \text{target plant}}{\text{Feeding on } C.odorata}$$

- **Feeding damage** reflects the relative damage determined using the mean percentage of damage per test species in proportion to that on *C. odorata* (relative feeding damage) in no-choice trials.

$$\text{Relative feeding damage} = \frac{\text{Feeding damage on non} - \text{target plant}}{\text{Feeding damage on } C.odorata}$$

2) Reproductive risk was calculated as survival of adults (relative survival) in no-choice tests, multiplied by the host suitability of test plants, and determined by the production of F_1 progeny (relative numbers) in no-choice tests (Mangan and Baars, 2023).

$$\textbf{Reproductive Risk} = \textbf{Adult survival} \times \textbf{Progeny production}$$

where:

- **Adult survival** is the relative suitability determined using the mean survival on the test plants in proportion to that on *C. odorata* as observed in no-choice trials.

$$\text{Relative survival} = \frac{\text{Survival rate on non} - \text{target plant}}{\text{Survival rate on } C.odorata}$$

- **Progeny Production** reflects the number of F_1 progeny produced on each test plant relative to those on *C. odorata*, as measured in no-choice trials.

$$\text{Relative progeny} = \frac{\text{Progeny on non} - \text{target plant}}{\text{Progeny on } C.odorata}$$

2.6. Host-specificity statistical analysis

All statistical analyses were conducted in the R environment version 3.5.2 (R_Core_Team, 2021). A binomial generalised linear mixed model (GLMM) with a logit link function was employed to examine the effects of different test plants and experimental runs on survival outcomes, incorporating a random intercept for each experimental run (1 | Run).. A Gaussian linear mixed model (LMM) with an identity link function was employed to examine the effects of different test plants and experimental runs on adult feeding, also including a random intercept for experimental variability (1 | Run). A generalised linear mixed model (GLMM) using a negative binomial distribution was employed, with glmmTMB function from the glmmTMB package in R (Brooks et al., 2017), to analyse the effects of different test plants and controlled for variability across experimental runs on total and plugged oviposition holes (1 | Run). A Poisson GLMM with a log link function was employed to examine the effects of different test plants and incorporated random effects to account for variability between different experimental runs on progeny production, specifically including a random intercept for each run (1 | Run). To facilitate direct comparisons with *C. odorata*, C. odorata was designated as the reference level for the Test plant variable. Fixed effects parameter significance was assessed using a Likelihood Ratio (LR) test ($p < 0.05$) using the 'car' R package (Fox and Weisberg, 2019). To facilitate the interpretation of the model coefficients, the raw beta coefficients were transformed into odds ratios for the binomial models and incidence rate ratios for the Poisson and Negative Binomial models. This transformation was performed by exponentiating the raw

coefficients (exp(coef(model)) in R).

To determine statistical differences during adult paired-choice tests, a Gaussian (GLM) with robust standard errors was employed to analyse the continuous variables 'position' and 'feeding'. The robust standard errors were calculated using the HC3 estimator to adjust for potential heteroscedasticity and provide reliable inference under the presence of unequal variances across groups. Count data for oviposition holes and progeny were analysed using Poisson regression models to address the distributional properties of count data. Initial assessments of over-dispersion were conducted by comparing the Pearson chi-squared statistic to the degrees of freedom of the model residuals. If significant overdispersion was indicated (Chi-squared/df > 1), a negative Binomial model was used to better accommodate the extra-Poisson variation. After fitting the model, an Analysis of Variance (ANOVA) using Type II sums of squares with a Likelihood Ratio (LR) test was conducted to determine the significance of differences among test plants using the 'car' package (Fox and Weisberg, 2019).

2.7. Native-range field plant inspection

Chromolaena odorata plants were inspected for the presence of adult weevils identical in appearance to those of the *L. aemulus* culture in South Africa. When the weevil or suspected adult weevil damage was present, all Asteraceae in the vicinity were inspected for the presence of adults of the same appearance as those on *C. odorata*. If possible, 10 plants of each species were examined per site. Plant height and the number of shoot tips (on which *Lixus* adults may be present) were estimated and grouped into one of six categories for each plant: 1 = 1–5; 2 = 6–10; 3 = 10–20; 4 = 20–50; 5 = 50–100; 6 > 100.

2.8. Insect collection

Adult weevils, similar in appearance to *L. aemulus,* were sampled from the Rio Branco area of Acre state, Brazil during field surveys (Supplementary Table S2). Adult weevils were collected at 3 sites where leaf damage was evident. Host plants included the target plant *C. odorata* as well as a species in the same genus, *C. laevigata*, and *H. vitalbae* on which characteristic *L. aemulus* adult weevil feeding damage was also evident. Additionally, individuals from the *L. aemulus* laboratory culture (originating in Rio Branco, Acre State) released as a biological control agent against *C. odorata* in South Africa in 2011, were collected. Additional sequences from GenBank were included in the analysis as outgroups: *Lixus filiformis* (Fabricius, 1781) (Coleoptera: Curculionidae) (host plant: *Picris* sp. ((Asteraceae)) and *Lixus angustatus* (Fabricius) (Coleoptera: Curculionidae) (host plant: *Carduus acanthoides* L. (Asteraceae)).

2.9. mtDNA extraction

All specimens were stored individually in 92 % ethanol. DNA was extracted from 14 adult *Lixus* (including five confirmed *L. aemulus*) using the Qiagen DNeasy Blood and Tissue kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's protocol. DNA quality and concentration were measured using a NanoDrop® ND-1000 Spectrophotometer (Labtech Int., UK). These extractions were then stored at −20 °C until required.

2.10. mtDNA sequencing

The cytochrome oxidase I (COI) region of the mitochondrial genome (mtDNA) was amplified for all samples. PCR reactions were carried out in 20 µl volumes under the following conditions: 4 µl of genomic DNA, 8.48 µl of ddH20, 1.8 µl of MgCl2 (25 mM), 0.6 µl of each primer (10 µM; Mtd6-curculio (5′ GGRGGWTTTGGAAAYTGAYTARTTCC 3′) and Mtd9-curculio (5′ CCNGGDARAATTAAAATRTMWACTTC 3′); (Simon et al., 1994), 0.4 µl dNTPs (10 mM each), 4.0 µl 10X buffer (Promega) and 0.12 µl of Taq polymerase. Thermal profiles started with an initial denaturing @ 94 °C for 60 s, followed by 32 cycles of 94 °C for 60 s, 50 °C for 45 s and 72 °C for 60 s. The cycle ended with one final extension of 240 s at 72 °C. The PCR products were purified, and sequencing reactions were conducted at Stellenbosch University DNA Sequencing Unit. Sequences were deposited in GenBank (Accessions PP054253 to PP054264).

2.11. mtDNA sequence analyses

Chromatogram contigs were assembled using CodonCode™ Aligner-Software (CodonCode Corp., Dedham, MA) and sequence alignments were proofread manually and aligned using Se-Al 2.0 (Rambaut, 2001). Phylogenetic analyses were conducted using MEGA 11 including the neighbour joining and maximum likelihood methods. The Tamura-Nei model and uniform rates of evolution assumption were employed for both methods (Tamura et al., 2011). Molecular Phylogenetic analysis by Maximum Likelihood tree using the GTR+G model of substitution of 3 control region haplotypes (411 bp). The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). The tree with the highest log likelihood (−2015.78) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach.

The analysis involved 14 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. There were a total of 411 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).

The haplotype diversity (h) mean pairwise differences (MPD) and nucleotide diversity (π) for each population were estimated using DnaSp. Ver. 5 (Librado and Rozas, 2009). Population structure was analysed using the Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992) and by calculating the FST values (Hudson et al., 1992) between populations, using the Kimura two-parameter distance method (Kimura, 1980). The statistical significance was determined by performing 1000 permutations of the original data set using Arlequin 3.0 (Excoffier et al., 2005).

2.12. Climatic suitability assessment ('climate matching')

The climate prediction-modelling program CLIMEX (Dymex Simulator: version 4.0.2) was used to compare the climatic similarity of the Brazilian collection sites of *L. aemulus,* namely Rio Branco, Acre State, Brazil, to Sub-Saharan Africa, more specifically the release sites of the weevil within South Africa. CLIMEX comparison between the Brazilian collection locality and South African release sites of *L. aemulus* was undertaken using the 'match climates' feature. The climatic parameters incorporated included mean annual rainfall, rainfall seasonality, minimum, maximum, and average temperature, relative humidity as well as soil moisture, all of which were equally weighted (=1) during the matching procedure. From these climatic matches, composite match indices were generated between the localities compared, typically collection vs release sites, with 0 % indicating no match and 100 % indicating a perfect match (Kriticos et al., 2015). In the case of biological control programmes, these climatic matches broadly offer insight toward the likelihood of agent establishment within the introduced range; with values of <50 % deemed as 'unsuitable', 50–59 % deemed as 'low', 60–69 % deemed as 'moderate', 70–79 % deemed as 'high' and ≥80 % typically being optimal (Cowie et al., 2023).

3. Results

3.1. No-choice adult survival, feeding, and oviposition

The analysis revealed significant differences in survival outcomes across the test plants ($\chi^2 = 565.24$, df = 31, $p < 0.0001$) and between experimental runs ($\chi^2 = 7.02$, df = 1, $p = 0.008$). These results indicate that the type of test plant significantly influenced survival, suggesting specific interactions between *L. aemulus* and certain plant species within the trial. The variation between runs also suggests possible effects of experimental conditions or temporal factors on mortality rates. *C. odorata, Ageratina riparia* (Regel) R.M. King & H. Rob. (Asteraceae) and *Bidens pilosa* L. (Asteraceae) supported the highest survival percentages among the test plants (Table 1). In the binomial logistic regression analysis, *C. odorata* was used as the reference plant against which the survival rates on other plants were compared. The results demonstrate a significantly higher probability of insect survival on *C. odorata*, with an odds ratio of 116.437. Conversely, the odds of survival on the alien ornamental species *Argyranthemum frutescens* (L.) Sch.Bip. and the invasive alien *Campuloclinium macrocephalum* (Less.) DC. (both Asteraceae) are substantially lower, with odds ratios of 0.006 and 0.009, respectively, both at a significance level of $p < 0.001$ (Supplementary Table S3). This indicates that the likelihood of insect survival is drastically reduced on these plants, being about 166.67 times lower on *A. frutescens* and about 111.11 times lower on *C. macrocephalum* compared to *C. odorata*. Analysis revealed significant differences in adult feeding across the test plants ($\chi^2 = 300.256$, df = 31, $p < 0.000001$). Almost all test plants exhibit significant differences in feeding behaviour relative to *C. odorata*, as indicated by the p-values mostly being less than 0.001. Adult weevils removed similar areas of leaf material on *C. odorata* and the invasive alien plant *Ageratina adenophora* (Spreng.) R.M.King & H.Rob. (Asteraceae). *Argyranthemum frutescens* and *A. hybridus* show large negative coefficients of −2.033 and −2.103 respectively, suggesting substantially reduced feeding, with significant p-values ($p < 0.001$) (Supplementary Table S4). Total oviposition holes were highest in *A. adenophora* and *Xanthium strumarium* L. (Asteraceae) (Table 1). The analysis revealed no significant differences in the number of oviposition holes across the test plants ($\chi^2 = 35.049$, df = 31, $p = 0.2818$). The vast majority of plant species show extremely negative coefficients (e.g., *A. frutescens, A. riparia*), suggesting a significantly lower number of oviposition holes compared to the host plant. However, the corresponding p-values are generally very high ($p = 0.999$), indicating that these differences are not statistically significant (Supplementary Table S5). The experimental run had no significant effect on the number of oviposition holes ($\chi^2 = 0.000$, df = 1, $p = 0.9830$). *Chromolaena odorata* obtained twice as many plugged holes (i.e., containing eggs) as the next two highest species, *A. conyzoides* and *B. pilosa.* About 70 % of holes bored by *L. aemulus* on *C. odorata* were plugged. In contrast, the number of probes on weed species such as *X. strumarium* was high, but the proportion of those chosen for egg-laying (i.e., plugged) was low (9.03 %). There was a strong dependency of progeny production on the type of test plant ($\chi^2 = 1617.1$, df = 31, $p < 0.0001$). *Chromolaena odorata* supported twice as much progeny production as non-target species. The host plant showed a positive and significant impact on progeny production, with an estimate of 32.385 and a significant p-value (<0.001) (Supplementary Table S6). Of the six non-target test species in which progeny were found, adults were obtained from all except weed *A. frutescens*.

3.2. Paired-choice adult position in cage, feeding, oviposition, and progeny production

In paired-choice trials, *L. aemulus* adults consistently favoured *C. odorata* over non-target species. During observations, *L. aemulus* adults were recorded 65–88 % of the time on *C. odorata* plants, 6–25 % on the five non-target species and 6–11 % elsewhere in the cage (Table 2). Adults spent the least amount of time on *C. macrocephalum*, *B. pilosa* and *Senecio madagascariensis Poir.* (Asteraceae). *Lixus aemulus* adults displayed a significant avoidance of *S. madagascariensis* for both position ($\chi^2 = 165.08$, df = 1, $p < 0.0001$) and feeding behaviours ($\chi^2 = 35.994$, df = 1, $p < 0.0001$) (Supplementary Table S7). All other paired-choice trials exhibited similar position and feeding behaviour trends, with significant differences in feeding amounts between the target and non-target test plant species.

Females bored more exploratory holes in *A. adenophora* than in *C. odorata*, but the number of these used for oviposition, i.e., plugged, was lower in *A. adenophora*, although not significantly so ($\chi^2 = 0.32335$, df = 1, $p = 0.5696$) (Table 3, Supplementary Table S7). For the four other test species, there were lower numbers of both exploratory and plugged holes than the corresponding control plants. Of the non-target species, *A. conyzoides* had the highest number of plugged oviposition holes, at 50 % of the number on *C. odorata*. *Senecio madagascariensis* and *B. pilosa* both had less than 10 % of the number of plugged holes recorded on *C. odorata*. Total and plugged oviposition activities did not significantly differ between the target and non-target plant species in any of the paired choice trials (Supplementary Table S7). *Ageratum conyzoides* produced 77 % of the progeny (larvae, pupae, adults) that the *C. odorata* control produced. The likelihood ratio tests revealed progeny production did not differ significantly ($\chi^2 = 0.11917$, df = 1, $p = 0.7299$). The other four non-target species produced less than 40 % of the number produced by their respective *C. odorata* controls. *Senecio madagascariensis* and *B. pilosa*, the only two species outside the Eupatorieae, contained the lowest numbers of progeny, with $\chi^2 = 15.545$ and 6.8231 (df = 1), which were both statistically significant ($p < 0.001$).

3.3. Risk assessment

The risk of feeding by *L. aemulus* on non-target plants is highest on *A. adenophora* and *A. conyzoides*, where the chance of sustaining damage relative to that of *C. odorata* was 29.05 and 6.44 % respectively when *C. odorata* is available in close proximity (Table 4). All other plants presented a less than 2 % chance of sustaining damage if growing close to *C. odorata. Ageratum conyzoides, A. adenophora* and *B. pilosa* present a reproductive risk of 46.08, 36.63, and 34 % respectively. In the absence of *C. odorata,* these plants are nutritionally suitable to produce progeny.

3.4. Native range field plant inspection

Four sites were inspected (RIOB 03, 06, 07, 08), of which *Lixus* sp(p). were present at three (not found at RIOB 07). Site size varied from 720-3000 m^2. All plants sampled were in the correct life stage and of an adequate size to realistically be attacked/utilised by the weevils. *Lixus* adults were found on three species of Asteraceae in Acre state, viz. *C. odorata*, *C. laevigata* and *H. vitalbae* (Table 5).

3.5. Sequence data, haplotypes and genetic diversity

After excluding the ambiguously called base pairs at the beginning and ends of each sequence, a 411-bp portion of the COI mtDNA sequences was obtained for 14 *Lixus* collected on *C. odorata* (n = 5), *C. odorata*: South African biotype (n = 5), *C. laevigata* (n = 3), and *H. vitalbae* (n = 1). A total of three different haplotypes were identified (Supplementary Table S8). *Lixus* sp. collected on *H. vitalbae* was the only population to contain a single haplotype. Haplotype diversity ranged from 0.400 (CO and COSA) to 0.667 (CL), with an average of 0.385 for all populations (Supplementary Table S9). Nucleotide diversity (π) ranged from 0.00105 (CO and COSA) to 0.00175 (CL), with an average of 0.00106.

3.6. Genetic relationships between populations

Three distinct clades were resolved by the mitochondrial CO1

Table 1
Adult no-choice feeding damage, survival, oviposition, and progeny production of the weevil *Lixus aemulus* exposed to plant species in no-choice conditions. See Table S1 for taxonomic details of plant species.

Test plant species	n	Adult feeding[a]	Relative damage[b]	% Survival[c]	Relative survival[d]	Total oviposition holes[e]	Plugged oviposition holes[e]	% plugged	Progeny	Relative progeny production[f]
Chromolaena odorata[w]	13	2.12 ± 0.77	1.0	93.38 ± 4.03	1.0	0.60 ± 0.30	0.42 ± 0.25	70.35	32.38 ± 19.24	1.0
Adenostemma caffrum[i]	3	0.79 ± 0.40	0.37	82.22 ± 9.35	0.88	0.30 ± 0.30	0.13 ± 0.02	8.41	0	0
Ageratina adenophora[w]	3	1.75 ± 0.55	0.83	92.68 ± 6.55	0.99	0.62 ± 0.45	0.13 ± 0.02	20.98	12.0 ± 16.52	0.37
Ageratina riparia[w]	3	0.43 ± 0.21	0.20	100.0 ± 0.00	1.0	0.06 ± 0.04	0.003 ± 0.01	4.79	0	0
Ageratum conyzoides[w]	3	0.98 ± 0.31	0.46	89.4 ± 9.36	0.96	0.52 ± 0.09	0.23 ± 0.07	43.40	15.67 ± 13.05	0.48
Campuloclinium macrocephalum[w]	3	0.57 ± 0.18	0.26	47.8 ± 41.40	0.51	0.10 ± 0.09	0.04 ± 0.04	41.32	3.67 ± 5.51	0.11
Mikania capensis[i]	3	0.15 ± 0.90	0.07	38.5 ± 36.50	0.41	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Stomatanthes africanus[i]	1	0.50	0.23	81.25	0.87	0.14	0.00	0	0	0
Helianthus annuus[c]	3	0.15 ± 0.07	0.07	37.74 ± 12.89	0.40	0.01 ± 0.01	0.00 ± 0.00	0	0	0
Helianthus tuberosus[c]	3	0.93 ± 0.71	0.44	48.90 ± 18.04	0.52	0.15 ± 0.13	0.10 ± 0.10	68.84	0	0
Xanthium strumarium[w]	3	1.18 ± 0.38	0.56	69.14 ± 45.7	0.74	0.80 ± 0.50	0.07 ± 0.07	9.03	0	0
Bidens pilosa[w]	3	0.80 ± 0.42	0.37	97.14 ± 4.95	1.0	0.29 ± 0.25	0.22 ± 0.20	73.35	11.33 ± 9.45	0.34
Bidens formosa[w, o]	3	0.00 ± 0.00	0.00	15.03 ± 16.9	0.16	0.06 ± 0.09	0.00 ± 0.00	0	0	0
Dahlia rosea[o]	3	0.02 ± 0.04	0.01	8.09 ± 7.01	0.09	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Chrysanthemum × *morifolium*[o]	3	0.33 ± 0.32	0.16	52.34 ± 17.0	0.56	0.003 ± 0.01	0.00 ± 0.00	0	0	0
Argyranthemum frutescens[o]	3	0.09 ± 0.90	0.04	31.2 ± 27.1	0.33	0.024 ± 0.03	± 0.01	18.09	1.0 ± 1.73	0.03
Symphyotrichum novi-belgii[o]	3	0.04 ± 0.07	0.02	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Senecio madagascariensis[i,w]	3	0.48 ± 0.27	0.22	70.94 ± 25.6	0.75	0.124 ± 0.15	0.09 ± 0.12	74.28	5.0 ± 4.36	0.15
Senecio tamoides[i]	3	1.26 ± 0.31	0.59	66.30 ± 14.3	0.71	0.11 ± 0.07	0.014 ± 0.01	12.94	0	0
Lactuca sativa[c]	3	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Cichorium intybus[c]	3	0.10 ± 0.17	0.05	3.03 ± 5.25	0.03	0.05 ± 0.08	0.00 ± 0.00	0	0	0
Distephanus angulifolius[i]	3	0.31 ± 0.12	0.14	42.5 ± 20.84	0.45	0.005 ± 0.01	0.002 ± 0.004	50	0	0
Gymnanthemum crataegifolium[i]	3	0.06 ± 0.05	0.03	11.07 ± 9.86	0.12	0.004 ± 0.01	0.004 ± 0.006	100	0	0
Cynara scolymus[c]	3	0.08 ± 0.04	0.04	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Ipomoea batatas[c]	3	0.01 ± 0.01	0.01	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Solanum lycopersicum[c]	3	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Amaranthus hybridus[c]	3	0.02 ± 0.03	0.01	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Beta vulgaris[c]	3	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Cucurbita pepo[c]	3	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00 ± 0.00	–	0	0
Phaseolus vulgaris[c]	3	0.05 ± 0.08	0.02	11.00 ± 19.2	0.12	0.00 ± 0.00	0.00 ± 0.00	–	0	0

n, Number of replicates.
[a] Adult feeding is expressed as leaf area (cm^2) removed per adult per day of the trial.
[b] Relative damage determined using the mean percentage of damage per test species in proportion to that on *C. odorata*.
[c] % of adults surviving over the 30 days.
[d] Relative suitability determined using the mean survival on the test plants in proportion to that on *C. odorata*.
[e] Oviposition holes are expressed as number per female per day of the trial.
[f] Relative progeny production determined using the mean progeny production on the test plants in proportion to that on *C. odorata*.

Table 2
Percentage of time spent by individuals and feeding damage of the adult *L. aemulus* during paired-choice tests.

Test plant	*n*	Position (% time)			Feeding (cm^2/adult/day)		Relative feeding
		C. *odorata*	Non-target	Elsewhere	*C. odorata*	Non-target	
Ageratina adenophora	3	0.65 ± 0.14a	0.25 ± 0.15b	0.10 ± 0.02	1.37 ± 0.42a	0.48 ± 0.23b	0.35
Ageratum conyzoides	3	0.69 ± 0.07a	0.23 ± 0.06b	0.08 ± 0.02	1.70 ± 0.40a	0.24 ± 0.12b	0.14
Bidens pilosa	3	0.88 ± 0.04a	0.09 ± 0.05b	0.08 ± 0.01	1.66 ± 0.01a	0.07 ± 0.03b	0.04
Campuloclinium macrocephalum	3	0.88 ± 0.04a	0.06 ± 0.06b	0.06 ± 0.03	1.84 ± 0.01a	0.01 ± 0.02b	0.01
Senecio madagascariensis	3	0.83 ± 0.10a	0.06 ± 0.03b	0.11 ± 0.09	1.83 ± 0.01a	0.01 ± 0.02b	0.01

Means (±SD) within columns followed by the same letter are not significantly different; Position: $P < 0.05$, Likelihood Ratio Test; Adult feeding: $P < 0.05$, Likelihood Ratio Test.
Test species are listed alphabetically.

Table 3
Oviposition and progeny production of the adult *L. aemulus* during paired-choice tests.

Test plant	*n*	No. oviposition holes/female/day				Progeny	
		Total		Plugged		*C. odorata*	Non-target
		C. odorata	Non-target	*C. odorata*	Non-target		
Ageratina adenophora	3	0.5 ± 0.1a	0.6 ± 0.4a	0.4 ± 0.1a	0.1 ± 0.1a	30.7 ± 14.6a	8.7 ± 4.3b
Ageratum conyzoides	3	0.6 ± 0.5a	0.4 ± 0.2a	0.5 ± 0.4a	0.2 ± 0.1a	14.3 ± 9.3a	11.0 ± 8.5a
Bidens pilosa	3	0.7 ± 0.3a	0.1 ± 0.1a	0.4 ± 0.1a	0.0 ± 0.0a	34.7 ± 26.2a	4.7 ± 4.5b
Campuloclinium macrocephalum	3	0.6 ± 0.5a	0.1 ± 0.2a	0.4 ± 0.4a	0.1 ± 0.1a	22.0 ± 5.6a	8.3 ± 12.7a
Senecio madagascariensis	3	0.3 ± 0.2a	0.1 ± 0.1a	0.3 ± 0.1a	0.02 ± 0.03a	13.0 ± 12.3a	0.3 ± 0.6b

Means (±SD) within columns followed by the same letter are not significantly different; Total and plugged oviposition: $P < 0.05$, Likelihood Ratio Test; Total number of progeny: $P < 0.05$, Likelihood Ratio Test.
Test species are listed alphabetically.

Table 4
Risk assessment of non-target attack by *L. aemulus*, using its preference for and performance on test plants in host-specificity trials relative to that on *C. odorata.*

Test plant species	Plant preference[a]	Feeding damage[b]	Feeding risk (%)[c]	Adult survival (%)[d]	Progeny production[e]	Reproductive risk (%)[f]
Chromolaena odorata[w]	1.0	1.0	100	1.0	1.0	100
Ageratina adenophora[w]	0.35	0.83	29.05	0.99	0.37	36.63
Ageratum conyzoides[w]	0.14	0.46	6.44	0.96	0.48	46.08
Campuloclinium macrocephalum[w]	0.01	0.26	0.26	0.51	0.11	5.61
Bidens pilosa[w]	0.04	0.37	1.48	1.0	0.34	34.00
Senecio madagascariensis[i,w]	0.01	0.22	0.22	0.75	0.15	11.25

[a] Attractiveness of test plant species, determined by adult preference (relative feeding) in paired-choice trials (Table 2).
[b] Host suitability of test plant species, determined by feeding damage (relative damage) in no-choice trials (Table 1).
[c] Product of suitability indices for preference [a] and performance [b].
[d] Host suitability of test plant species, determined by adult survival in no-choice trials (Table 1).
[e] Host suitability of test plant species, determined by the production of progeny (relative progeny production) in no-choice trials (Table 1).
[f] Product of suitability indices for adult survival [d] and production of progeny [e].

Table 5
Plants examined in Acre state, Brazil, for the presence of *Lixus* sp(p). Adults. Means ± SD are given. See Table S1 for taxonomic details of plant species.

Plant species	n[1]	No of *Lixus* sp(p). per plant	Total *Lixus*	No. of plants with *Lixus* (%)
Chromolaena laevigata	30 (3)	0.27 ± 0.69	8	5 (16.7)
Chromolaena odorata	29 (4)	0.21 ± 0.49	6	5 (17.2)
Clibadium surinamense	1(1)	0	0	0
Heterocondylus vitalbae	9(2)	0.33 ± 0.71	3	2 (22.2)
Praxelis clematidea	10 (1)	0	0	0
Tilesia baccata	11 (2)	0	0	0
Tithonia diversifolia	1(1)	0	0	0
Vernonanthura patens	28 (4)	0	0	0

Test species are listed alphabetically.
[1] No. individual plants (no. sites).

sequence analysis for 18 specimens, representing three different species on a variety of host plants. One clade represents specimens collected on *C. odorata* (both during the current survey and from the South African laboratory culture), *C. laevigata,* and *H. vitalbae* during the native-range exploration. Another clade denotes the outgroup *L. filiformis* and the final clade represents *L. angustatus* (Fig. 2). The K2P genetic distances between haplotypes within the 6 populations ranged from 0.001 (*L. aemulus* on *C. odorata* (SA biotype), *Lixus* sp. on *C. odorata*, *C. laevigata,* and *H. vitalbae*) to 0.353 (*L. angustatus* on *Picris* sp.) (Table 6). The overall mean within population was 0.133. Mean distances between populations ranged from 0.001 between *L. aemulus* on *C. odorata* (SA biotype) and *Lixus* sp. on *C. laevigata* to 0.353 between *Lixus* sp. on *C. laevigata* and *L. angustatus* on *Picris* sp. An AMOVA was performed on the 14 specimens of *Lixus* sp(p). collected in Brazil and *L. aemulus* from *C. odorata* (South African biotype) demonstrated that 95 % ($\Phi_{ST} = 0.9518$, $P > 0.01$) of the variance occurred within populations (Supplementary Table S10). AMOVA also established low variance between populations ($\Phi_{ST} = 0.0482$, $P < 0.01$) indicating a lack of genetic structure.

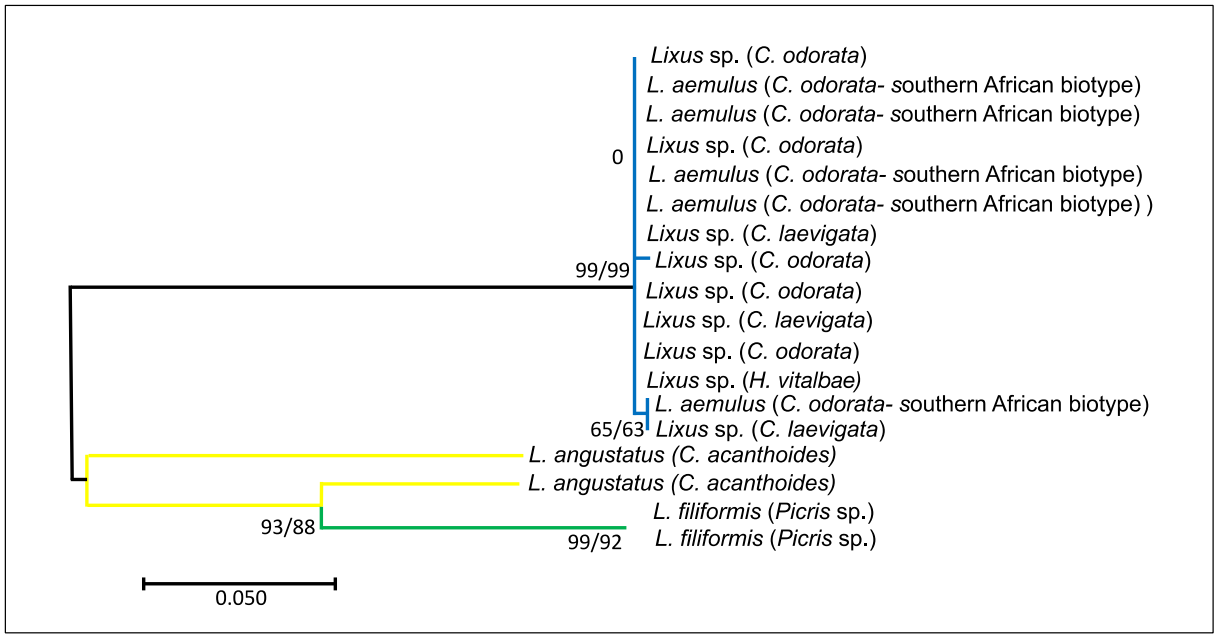

Fig. 2. Maximum Likelihood tree using the GTR+G model of substitution of 3 control region haplotypes (411 bp) resolving three major lineages; *Lixus aemulus* samples are indicated in blue, *Lixus angustatus* are indicated in yellow, and *Lixus filiformis* are indicated in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Pairwise F_{ST} values between (below diagonal) and within (diagonal) *Lixus* sp. populations based on the Kimura two-parameter distance between mtDNA haplotypes.

Species (host plant)	*L. aemulus (C. odorata*)*	*Lixus* sp. *(C. odorata)*	*Lixus* sp. *(C. laevigata)*	*Lixus* sp. *(H. vitalbae)*	*L. filiformis (C. acanthoides)*	*L. angustatus (Picris* sp.)
L. aemulus (C. odorata)*	0.001					
Lixus sp. *(C. laevigata)*	0.001	0.002				
Lixus sp. *(C. odorata)*	0.001	0.001	0.001			
Lixus sp. *(H. vitalbae)*	0.001	0.001	000.1	n/c		
L. filiformis (C. acanthoides)	0.328	0.328	0.327	0.327	0.011	
L. angustatus (Picris sp.)	0.353	0.353	0.353	0.353	0.233	0.261

FST values range from 0.0 (no differentiation) to 1.0 (complete differentiation).
n/c: not enough replicates to conduct within haplotype diversity.
* Southern African biotype.

3.7. Climatic suitability assessment ('climate matching')

Overall, much of Sub-Saharan Africa remains poor to moderately matched climatically to the Brazilian collection sites of *L. aemulus,* with only the western and central tropical regions presenting high (>70 %) to optimal (>80 %) climatic matches (Fig. 3A). As for South Africa, the climatic parameters for all the release sites of *L. aemulus* showed a mean match of 58 ± 2 % to the native Brazilian collection sites (Supplementary Table S11). Climatic matching indicated that rainfall and temperature showed the greatest discrepancies amongst the climatic variables between the collection and release sites, particularly for most inland regions of South Africa. Release sites of *L. aemulus* located along the KwaZulu-Natal coastline maintained the highest composite match indices (climate matches) to Rio Branco, Brazil, ranging between low to moderate, with matches of 59–66 % (Fig. 3B). The remaining release sites in the Mpumalanga and Limpopo provinces were poorly matched to the Brazilian collection sites, averaging low matches of 52 % and 50 % respectively (Fig. 3B).

4. Discussion

A total of 29 test plant species, consisting mainly of Asteraceae but including a few non-asteraceous crop species, was used for testing the laboratory host specificity of *L. aemulus.* The preferred host of *L. aemulus* is C. *odorata*, under both no-choice and paired-choice conditions in cages in the laboratory. The ovipositing female is the host-selecting life stage, which is typical of insect species whose larvae are endophagous, and have 'limited mobility' (Prager et al., 2014). The female bores an exploratory oviposition hole, and only lays her egg if she finds the host suitable. Once the egg has been laid it usually hatches (87.4 % (n = 103) for which this was recorded) and the progeny develops through to adulthood. The plant species must be physically suitable before oviposition occurs. Stems must have a pithy centre for the larva to survive and they must be of sufficient diameter. Although the adults were able to feed and survive on a range of Asteraceae under no-choice conditions, they caused negligible damage. Adult females were selective of the species in which they laid eggs. Larval progeny were obtained in six of the twenty-nine non-target species exposed to *L. aemulus* (*A. frutescens, A. adenophora, A. conyzoides, B. pilosa, C. macrocephalum, S. madagascariensis*) and adult progeny in five (all but *A. frutescens*). Increased selectiveness was evident under paired-choice conditions. No crop species from other families on which other species of *Lixus* have been recorded as pests were suitable for the survival of *L. aemulus.*

Native-range surveys confirmed that the weevil was present on *C. odorata*. An equivalent number of *Lixus* adults were found on C. *laevigata* and *H. vitalbae*. Several other species of Asteraceae surveyed had

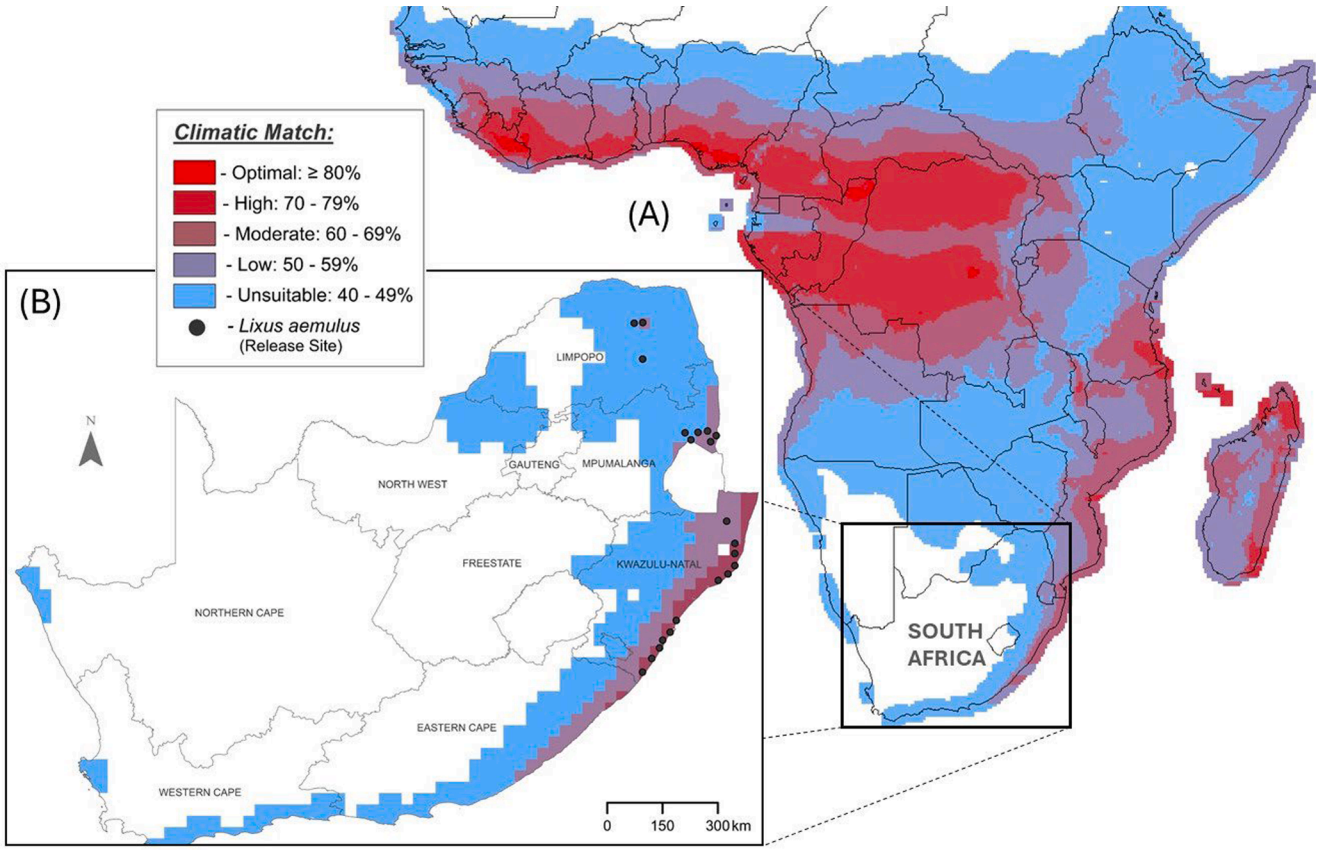

Fig. 3. Projected climatic match of Lixus aemulus collection sites in Rio Branco, Acre state, Brazil to (A) Sub-Saharan Africa, (B) South African release sites. Resolution of grid cells for South Africa is at a quarter degree square (QDS: ~25 km × 25 km) (data adapted from CLIMEX: Kriticos et al. (2015)).

no adults present. Molecular analysis reveals low levels of genetic diversity and low levels of genetic differentiation across the individuals collected on *C. odorata* (southern African biotype), *C. odorata* (in the native range), *C. laevigata* and *H. vitalbae* for mitochondrial markers. The low levels of genetic diversity observed appear to be correlated with low Fst and $\Phi_{ST,}$ suggesting low differentiation between populations of *L. aemulus and Lixus* sp(p). This indicates that the weevils across all three plant species collected in Brazil in 2015, as well as the SA laboratory culture, collected opportunistically from a pubescent 'hairy chromolaena' in 1995 (Kluge and Zachariades, 2006), belong to one interbreeding population and they are all one species, identified by C. O'Brien as *L. aemulus* or near. This also confirms the results of laboratory trials conducted in quarantine that indicate that *L. aemulus* is not monophagous. The host range of *L. aemulus* is confined to certain plants within the tribe Eupatorieae, and therefore it still has an acceptably narrow host range for safe release in South Africa (Zachariades et al., 2021). In Rio Branco, only adults and their non-target feeding damage were found on *C. laevigata* and *H. vitalbae.* These plants may not have been used as a larval host plant and laboratory host-range trials indicated that adults feed on a broader range of species than the females oviposit on. However, it seems unlikely that these two species were not acting as larval host plants as adult *L. aemulus* are quite sedentary and feeding and mating on both plant species, which were about 150 m from the closest *C. odorata* plants.

Permission to release *L. aemulus* in South Africa was obtained in 2010, and releases of over 5,500 adults were made at 21 sites between 2011 and 2019. However, establishment of the weevil appears to have been limited. In addition to concern surrounding the partial or complete clearing of some ~20 % of *L. aemulus* release sites by 2016 (Zachariades et al., 2021), much of South Africa's climate was found to be poorly matched to Rio Branco, Brazil. Considerable climatic mismatch, most notably in rainfall and temperature, between South African regions invaded by *C. odorata* and the collection sites of *L. aemulus* is likely to have constrained the establishment and proliferation of the weevil (Robertson et al., 2008). Hindrances to establishment, due to climatic mismatches, have been a common occurrence in South African biocontrol programmes, particularly when agent collections have occurred in high-rainfall, tropical regions of South America (Cowie et al., 2016). Often substantial mismatches like this result in the persistence of agents at low abundances, typically in localised to smaller areas, or more severely a complete failure to establish (Cowie et al., 2016; Harms et al., 2021). Broadly matching the South African regions invaded by *C. odorata* to South America may offer useful insight for potential collections in climatically better suited localities, with preliminary matching suggesting that semi-arid regions, such as Remanso and Caetité, in the State of Bahia, appear as more suitable collection localities. However limited information regarding the distribution of *L. aemulus* in Brazil may pose difficulties in assessing whether this agent could in fact be collected from these regions (Zachariades et al., 2021).

Although climate remains one of the major constraints to biological control programmes (Harms et al., 2021), with climatic mismatches sought to be avoided, the biology of oligophagous species used for biological control endeavours should also be taken into consideration. Oligophagous species, such as *L. aemulus,* often display differential feeding and performance amongst suitable host plants, as seen in this study, which offers the possibility that the weevil may perform suboptimally on the southern African biotype of *C. odorata*. *Chromolaena odorata* varies considerably in morphology (growth habit, leaf and stem pilosity, inflorescence structure and colour, etc.) as well as the odour of its crushed leaves (indicating chemical differences) across its native range, and the two major invasive biotypes differ from one another in ecological characters such as susceptibility to burning and their

performance at a given ambient temperature (Zachariades et al., 2009).

Lixus aemulus was collected opportunistically from a tropical region with high rainfall at a time when the origin of the *C. odorata* population invading southern Africa was unknown. Although the weevil was shown to be adequately damaging and suitability host-specific for release within South Africa, its poor establishment thus far justifies concerns regarding climatic and biotype mismatches. This situation advocates for the prioritisation of biological control agents from regions of the native range of *C. odorata* that are climatically more similar to the invaded areas in South Africa.

5. Author statement

Host specificity: Laboratory: C.Z. conceived the experiments, C.Z. and M.G. completed the laboratory work; field: C.Z. and M.B. recorded *L. aemulus* field-range data, C.G.S., M.S. and A.M.T. assisted in the identification of surveyed Asteraceae; R.M. and C.Z. conducted the analysis and prepared the manuscript. All authors provided critical feedback and helped shape the final manuscript.

mtDNA: R.M. and C.Z. conceived the experiments, R.M. contributed to sample preparation, conducted the analysis and prepared the manuscript. All authors provided critical feedback and helped shape the final manuscript.

Climatic matching: B.C., R.M., and C.Z. conceived the experiments. B.C. conducted the analysis. R.M., C.Z. and B.C prepared the manuscript. All authors provided critical feedback and helped shape the final manuscript.

CRediT authorship contribution statement

Rosie Mangan: Writing – review & editing, Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Milly Gareeb:** Writing – review & editing, Data curation. **Marcus Boeno:** Writing – review & editing, Data curation. **Chirley Gonçalves da Silva:** Writing – review & editing, Data curation. **Blair Cowie:** Writing – review & editing, Methodology, Formal analysis. **Aristônio Magalhães Teles:** Writing – review & editing, Data curation. **Marcos Silveira:** Writing – review & editing, Data curation. **Costas Zachariades:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2024.105591.

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