




Exploring the diversity of leafrollers (Lepidoptera: Tortricidae) associated with eucalypts in South African forestry nurseries and plantations, with a new report of *Strepsicrates* sp.

Privilege T Makunde¹ , Bernard Slippers²  and Brett P Hurley¹ 

¹ Department of Zoology and Entomology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

² Department of Biochemistry, Genetics and Microbiology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, 0002, South Africa

* Corresponding author: privilege.makunde@fabi.up.ac.za

A number of insect pests cause damage to eucalypts in South Africa. Their accurate identification is a key part of managing and controlling these pests. The aims of this study were to identify leafroller insect species in South African eucalypt nurseries and plantations, and to determine whether the species were native insects that had developed new host associations with eucalypts or introduced non-native species. A section of the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene of multiple specimens from nurseries and plantations was sequenced. The sequence data were compared and validated using sequences available in BOLD systems and GenBank databases. Results revealed the presence of an introduced non-native *Strepsicrates* sp. in both nurseries and plantations. In addition, the native species *Choristoneura occidentalis* and *Eccopsis incultana* were confirmed as present in plantations only and the native species *Lozotaenia capensana* was confirmed as present in one of the nurseries. Eucalypts are reported as host plants of *C. occidentalis*, *E. incultana* and *Lozotaenia capensana* for the first time. The findings contribute valuable insights into the identification and diversity of leaf rollers in eucalypt plantations in South Africa, with potential implications for pest management.

Key words: CO1, pest management, Tortricidae, *Strepsicrates*, *Eucalyptus*

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Introduction

Eucalypts (Myrtales: Myrtaceae) are a highly diverse genus comprised of 10 subgenera, with approximately 730 species (Brooker 2000; Nicolle 2022). These evergreen trees are native to Australia, Papua New Guinea (including New Britain), Timor-Leste, Indonesia and the Philippines (Nicolle 2022). Among the 10 subgenera, *Symphomyrtus* is the dominant subgenus in global plantation forestry, grown for various purposes (Beadle and Turnbull 1992). In South Africa, eucalypts have a pivotal role in the forestry industry, occupying approximately 49.4% of the nation's 1.2 million hectares of land dedicated to forestry activities (Morris 2022).

As with other countries where eucalypts are planted as non-natives, a number of eucalypt feeding insects native to Australia have been accidentally introduced to South Africa over time (Hurley et al. 2016; Paine et al. 2011; Wingfield et al. 2020). As of 2017 there were 11 non-native insect pests of eucalypts reported in South Africa (Hurley et al. 2017). In addition, a number of native insects developed new host associations with the exotic eucalypts (Hurley et al. 2017; Wingfield et al. 2020). Some of these, such as the introduced *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae) and *Gonipterus* sp. n. 2 (Coleoptera: Curculionidae) and the native *Coryphodema tristis* Drury (Lepidoptera: Cossidae) have become serious pests of eucalypts in South

Africa, requiring costly management interventions such as breeding for resistance, biological control and mass trapping (Dittrich-Schröder et al. 2012, 2014; Noeth et al. 2020; Schröder et al. 2020; Wingfield et al. 2020).

Since 2019, leafroller insects (Lepidoptera: Tortricidae) have been reported feeding on various eucalypt species and hybrids in a number of commercial nurseries and plantations in South Africa. These were the earliest reports in South Africa of leaf rollers causing damage on eucalypts, so the identification of the species involved and thus their potential importance as pests was hitherto unknown. The family Tortricidae contains some significant pests in agriculture and forestry (Mauchline et al. 1999; Timm 2005; Wakamura et al. 2005; Brown et al. 2008). *Strepsicrates* spp. are significant pests in eucalypt plantations, especially in Southeast Asia and China (Zhenghong 2003; Kkadan et al., 2020; Srikumar et al., 2022). In these regions, *Strepsicrates semicanella* in particular is known to cause substantial damage to young eucalypt plants, leading to reduced growth and economic losses. Their larvae damage plants by consuming shoot tips, buds, and developing flowers, encasing them in webbing inside a rolled leaf, ultimately causing leaf withering and death (Phillips 1992; Zhenghong 2003; Srikumar et al., 2022).

In this study, we used cytochrome *c* oxidase subunit I mitochondrial (CO1) mitochondrial sequence data to identify the leaf roller species affecting eucalypts in South Africa. DNA barcoding is a widely acknowledged tool for identification and addressing taxonomic issues in Lepidoptera, and numerous instances of its application are reported within the Tortricidae (Brown et al. 2014a; Gilligan et al. 2016; Escobar-Suárez et al. 2017). We further assessed whether the same species were involved in both the nurseries and the eucalypt plantations, and whether the species were native insects that had developed new host associations with eucalypts or introduced non-native species.

Materials and methods

Leaf roller collection and preservation

Leafroller larvae collected from nurseries and plantations from March 2019 to November 2023, as well as samples collected from landowners and sent directly to the FABI Diagnostic Clinic in the same period, were used in this study. A total of 100 larvae were obtained from the four eucalypt growing regions in the country, namely Limpopo, KwaZulu-Natal, Mpumalanga and Western Cape provinces (Figure 1; supplementary Table S1). Host records for eucalypt-leaf roller association were recorded (supplementary Table S1). All collected samples were preserved in 70% ethanol and stored at -20°C until used for molecular analysis. All leafrollers obtained in this study are preserved in the insect collection at the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa.

DNA extraction

DNA was extracted from 100 leafroller larvae, including 52 from KwaZulu-Natal, 14 from Limpopo, 22 from Mpumalanga, and 12 from Western Cape. Specimens preserved in 70% ethanol were rinsed in sterile distilled water to remove excess ethanol, before DNA extraction. Total genomic DNA extraction from two front legs of individual specimens were performed using a *prepGEM*[®] Tissue Kit (ZyGEM) following the manufacturer's instructions with the following modification. The reaction mixture per individual sample DNA extraction was adjusted to 25 μl (20 μl ultrapure

(SABAX) water, 4 μl 10 \times BLUE buffer and 1 μl *prepGEM*). For qualitative testing, 5 μL of DNA samples were resolved by gel electrophoresis in 2% agarose gel in SB (1 \times) buffer at 80V (45 min) and visualised using a BioRad Gel Doc[™] EZ Imager and the software Image Lab v4.0 build 16. One (1) kb Plus DNA Ladder (Invitrogen) was run in parallel with the samples. DNA concentration was measured using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, U.S.A) and adjusted to 2 ng μl^{-1} .

PCR amplification of the CO1 were performed using LepF1: 5' ATTCAA CCA ATCATA AAG ATATTG G 3', and LepR1: 5' TAA ACT TCTGGA TGCCA AAA AATCA 3' (Hebert et al. 2004) to yield a 658 bp fragment. All PCRs were performed in a final volume of 25 μl in 0.2 ml Eppendorf tube containing 0.5 μL of DNA template, 5 μl MyTaq buffer, 0.5 μl 2.5 u / 50 μl) of MyTaq DNA polymerase, 1 μl (10 pmol) of each primer (LepF1 and LepR1), and 17 μl sterilised distilled water. The PCR reactions were run on an Eppendorf Mastercycler[®] pro thermocycler following the protocol described by Hebert et al. (2004). The PCR cycling parameters were as follows: an initial denaturation step at 94°C for 1 min, denaturation at 94°C for 1 min, annealing at 45°C for 1 min and 30 sec, extension at 72°C for 1 min and 15 sec (6 cycles), denaturation at 94°C for 1 min, annealing at 51°C for 1 min and 30 sec, extension at 72°C for 1 min and 15 sec (36 cycles), and a final extension at 72°C for 5 min. Successful amplifications of the CO1 gene region were verified by staining 2 μl of each PCR product with 1 μl GelRed[™] (Biotium, USA) nucleic acid dye and were resolved in 2% SB (1 \times) buffered agarose mini-gel at 100 V (40 min) along a molecular weight marker (100 bp Ladder, Invitrogen). The products were visualised using a Bio-Rad Gel Doc[™] EZ Imager and the software Image Lab v4.0 build 16.

Cleaning PCR products and sequencing PCR

The PCR products were cleaned by an ExoSAP-IT (USB Corporation, Cleveland, OH) treatment; where 8 μl of ExoSAP-IT is added to each PCR product and incubated at 37°C and 80°C , for 15 min at each temperature, with a final incubation at 4°C hold. The forward and reverse sequencing reactions were performed in a 12 μl reaction volume made of 7.5 μl of ultrapure (SABAX) water, 1.0 μl of sequencing buffer, 1.0 μl of BigDye[™] Ready Reaction Mixture with

Table 1: *Strepsicrates* species DNA barcode sequences (632 bp) of the mitochondrial gene section, cytochrome oxidase subunit I used in the phylogenetic analysis

Species	BOLD accession	GenBank accession	Country
<i>Strepsicrates</i> sp.	N/A	N/A	South Africa
<i>Strepsicrates</i> sp.	MIMAD017-15	MH418398.1	Madagascar
<i>Strepsicrates semicanella</i>	ANICW367-11	KF405198.1	Australia
<i>Strepsicrates dyselia</i>	ANICC242-09	GU688770	Australia
<i>Strepsicrates transfixa</i>	ANICW377-11	KF397383.1	Australia
<i>Strepsicrates macropetana</i>	ANICC262-09	GU688756.1	Australia
<i>Choristoneura occidentalis</i>	AFTOR002-12	KJ592330.1	Kenya
<i>Choristoneura occidentalis</i>	N/A	N/A	South Africa
<i>Eccopsis incultana</i>	AFTOR278-12	N/A	Nigeria
<i>Eccopsis incultana</i>	N/A	N/A	South Africa
<i>Lozotaenia capensana</i>	N/A	OQ836383.1	South Africa
<i>Lozotaenia capensana</i>	N/A	N/A	South Africa

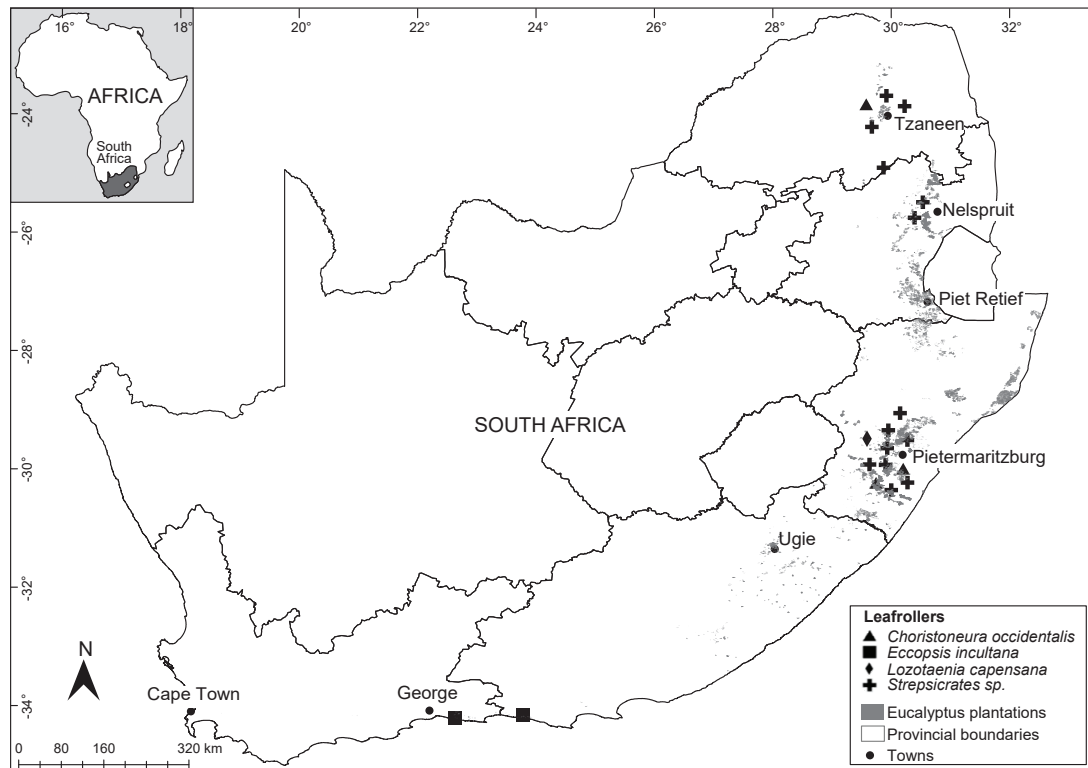


Figure 1: Current distribution leafrollers in South African eucalypt nurseries and plantations

Amplitaq DNA polymerase (Perkin-Elmer Applied Biosystems, Warrington, UK), 0.5 μl of either of the primers (10 μM) and 2 μl of purified PCR product (50 $\text{ng } \mu\text{l}^{-1}$). The reaction mixtures were then incubated on an Eppendorf Mastercycler® Pro thermocycler at an initial denaturation step for 2 min at 96 °C, 30 subsequent cycles at 96 °C for 30 s, 54 °C for 15 s and 60 °C for 4 min. The sequencing products were cleaned by Ethanol/NaAC precipitation of BigDye Terminator v3.1 DNA sequencing reactions protocol from the ABI manual; were 50 μl of 99% ethanol, 2 μl of sodium acetate (3M) and 8 μl of ultrapure (SABAX) water are added to a final volume of 72 μl . The mixture was incubated at -20 °C for 10 min, centrifuged for 30 min at 14 000 rpm and washed twice with 150 μl of 70% ethanol at 14 000 rpm discarding the supernatant at each run. The pellets of the sequencing PCR amplicons were vacuum dried and concentrated using Eppendorf Concentrator 5301 at 60 °C for 20 min. Sequencing of the PCR products was performed on an ABI Prism™ 3100 Genetic Analyzer (Applied BioSystems, USA).

Molecular analysis

The DNA sequences were edited using CLC Main Workbench 6.0 (CLC Bio, Denmark) and Biological Sequence Alignment Editor (BioEdit) software (Hall 1999) version 7.0.9. Molecular identification consisted of sequenced comparisons with the Barcode of Life Data Systems (BOLD) (Ratnasingham and Hebert 2007) and using the Basic Local Alignment Search Tool (BLAST, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) for CO1. In order to enhance and confirm the generated sequences, we incorporated CO1 sequences from Barcode of Life Data Systems

(BOLD) (Ratnasingham and Hebert 2007) and GenBank databases for the relevant species into our analyses. We retrieved 658 bp DNA barcode sequences for the Eucosmini tribe (*Strepsicrates*). These sequences were selected as congeneric representatives and they encompassed a range of species within *Strepsicrates*, namely *Strepsicrates dyselia* (Turner), *S. semicanella* (Walker), and *S. transfixa* (Turner) (Table 1). *Choristoneura occidentalis* Lederer and *Lozotaenia capensana* Walker were included as representatives of the sister tribe Archipini, and *Eccopsis incultana* (Walker) as a representative of the sister tribe Olethreutini (Table 1). All DNA barcode sequences were aligned using Multiple Sequence Alignment Program (MAFFT) version 7 (<http://mafft.cbrc.jp/alignment/software/>) (Kato and Standley 2013). The aligned sequences were further edited in BioEdit by comparing against sequencing trace files. The phylogenetic relationship amongst the leafrollers was inferred using the Maximum Likelihood method and General Time Reversible model (Nei and Kumar 2000), with 1 000 bootstrap replicates using the MEGA11 (Tamura et al. 2021). Estimates of evolutionary divergence among *Strepsicrates* sp. (South Africa), *S. dyselia*, *S. semicanella*, *S. transfixa*, *C. occidentalis*, *E. incultana* and *L. capensana* was computed using the Kimura 2-parameter model in MEGA11 software (Kimura 1980; Tamura et al. 2021).

Results

Using DNA barcode sequences of the mitochondrial gene (*COI*) from 100 samples, we identified four distinct leafroller species (tortricids) feeding on eucalypts: an

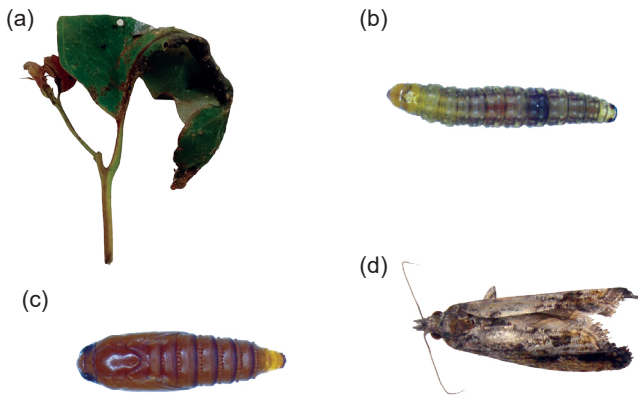


Figure 2: (a) Rolled eucalypt leaf with larva of *Strepsicrates* sp.; (b) *Strepsicrates* sp. larva; (c) *Strepsicrates* sp. pupa; (d) a moth of the eucalypt leaf roller, *Strepsicrates* sp.

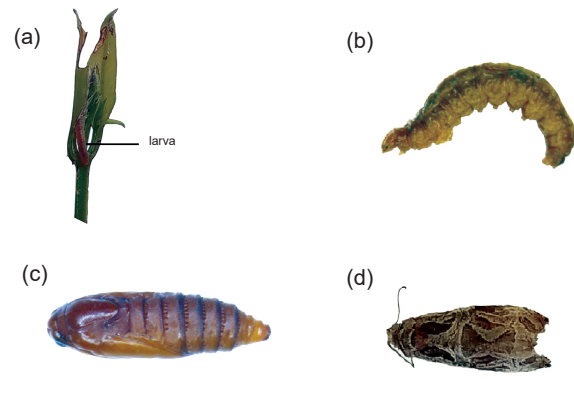


Figure 3: (a) Rolled eucalypt leaves that have been partially opened to reveal the larva of *Eccopsis incultana*; (b) *E. incultana* larva; (c) *E. incultana* pupa; (d) *E. incultana* moth

Table 2: Leafroller species composition of eucalypt nurseries and plantations in different provinces of South Africa

Source	Species	Province	No. of larvae	Lab ID
Nurseries	<i>Strepsicrates</i> sp.	KwaZulu-Natal	30	LR001 – 30
		Limpopo	9	LR031 – 39
		Mpumalanga	11	LR040 – 50
Plantations	<i>Strepsicrates</i> sp.	KwaZulu-Natal	12	LR051 – 62
		Limpopo	5	LR063 – 67
		Mpumalanga	7	LR068 – 74
Plantations	<i>Choristoneura occidentalis</i>	KwaZulu-Natal	10	LR075 – 84
Nursery	<i>Lozotaenia capensana</i>	KwaZulu-Natal	6	LR085 – 90
Plantations	<i>Eccopsis incultana</i>	Western Cape	10	LR091 – 100

unknown *Strepsicrates* sp. (Lepidoptera: Tortricidae) (74), *Choristoneura occidentalis* Lederer (Lepidoptera: Tortricidae) (10), *Eccopsis incultana* (Walker) (Lepidoptera: Tortricidae) (10) and *Lozotaenia capensana* Walker (Lepidoptera: Tortricidae) (6) (Table 2). The previously unknown *Strepsicrates* sp. was confirmed in nurseries and plantations in multiple locations, including Limpopo, KwaZulu-Natal, and Mpumalanga provinces, on *E. dunnii*, *E. grandis*, *E. radiata*, *E. grandis* × *urophylla* (GU) and *E. grandis* × *nitens* (GN) hybrids (Figure 1; Table 2). Associated leaf malformation and life stages (larval, pupal, and adult) are illustrated in Figure 2. In contrast, *C. occidentalis* was confirmed in two plantations in KwaZulu-Natal and Limpopo (Figure 1) on a GN hybrid, while *E. incultana* was only confirmed in two plantations situated in the Western Cape on *E. grandis*, with associated leaf malformation and life stages depicted in Figure 3. *Lozotaenia capensana* was only confirmed in one nursery located in KwaZulu-Natal on *E. grandis* (Figure 1; Table 2). We were unable to obtain the adult stages (moths) for two of the leafroller species, *C. occidentalis* and *L. capensana*.

To explore the genetic relationships among the leafroller species, we conducted a phylogenetic analysis based on the barcoding section of the *COI* mitochondrial DNA sequences. As no variation was observed in the sequences of *Strepsicrates* spp., *C. occidentalis*, *E. incultana*, and

L. capensana, one reference sequence was used for each species in the phylogenetic tree. The analysis showed three separate clades: one corresponding to *C. occidentalis* and *L. capensana*, and the other two representing *Strepsicrates* and *E. incultana* (Figure 4). The pairwise distance (K2P) between the two *Strepsicrates* spp., one from the nurseries and the other one from plantations, was 0%, with divergence from congeneric species ranging from 4.1% (*S. semicanella*) to 4.9% (*S. dyselia*) (Supplementary Table 2). In comparison to other tortricids from the sister tribes *Archipini* and *Olethreutini*, sequence divergence ranged from 8.9% (*L. capensana*) to 10.9% (*C. occidentalis*). Additionally, the *E. incultana* sequence from South African plantations diverged by 0.3% from the *E. incultana* (Nigeria) DNA barcode sequences from BOLD. Similarly, the divergence between *C. occidentalis* (South Africa) and *C. occidentalis* (Kenya) in BOLD was 0.6%, and the *L. capensana* found in a South African *Eucalyptus* nursery diverged by 2.1% from *L. capensana* (South Africa) (Supplementary Table 2).

Discussion

Utilising DNA barcoding, we investigated the diversity of leafrollers collected from both nurseries and plantations of eucalypts in South Africa. Through this analysis, we identified

a new *Strepsicrates* sp. in both nurseries and plantations, and in one nursery *Lozotaenia capensana*, and in plantations only *Choristoneura occidentalis* and *Eccopsis incultana*. The *Strepsicrates* sp. was confirmed to be an introduced non-native species, but different to the known *Strepsicrates* spp., namely *S. macropetana* and *S. semicanella*, which have become invasive pests in other countries. *Lozotaenia capensana*, *C. occidentalis* and *E. incultana* were confirmed as insects native to South Africa. This is the first report of a new *Strepsicrates* sp., as well as of the above-mentioned native leaf roller species feeding on *Eucalyptus* spp., in South Africa.

The presence and widespread distribution of an unknown introduced *Strepsicrates* sp. in eucalypt nurseries and plantations in South Africa is of particular concern. The genus *Strepsicrates* Meyrick (Lepidoptera: Tortricidae: Olethreutinae: Eucosmini) has a widespread distribution. It is especially common in Australia and other Oceania regions (Horak 2006; Razowski 2016). *Strepsicrates* larvae feed on host plants in the Myricaceae and Myrtaceae families and a number of *Strepsicrates* species are considered economically important pests in agriculture and forestry (Mauchline et al. 1999; Wakamura et al. 2005). A notable

example in forestry is *S. semicanella*, which is considered highly invasive and causes substantial damage (Nair 2001; Nasu et al. 2004; Razowski 2013; Srikumar et al. 2022). In Africa *Strepsicrates routhia* (Meyrick) (syn. *Spilonota routhia*) and *Strepsicrates sinuosa* (Meyrick) (syn. *Spilonota sinuosa*) have been reported (Razowski 2015; Williams and Ranwashe 2016), but their economic significance is unknown. The *Strepsicrates* species identified in South Africa is genetically similar but distinct from *S. semicanella*, and thus the host range, beyond what is reported in this study, and the potential pest status, is currently unknown.

The impact of *Strepsicrates* spp. on eucalypt plantations in regions like Southeast Asia and China is well-known (Zhenghong 2003; Kkadan et al., 2020; Srikumar et al., 2022). For instance, in Indonesia, *S. semicanella* significantly reduces the growth of young eucalypt plants, and control measures such as the use of spinetoram 120 SC with adjuvants have proven effective despite challenging environmental conditions, such as heavy rainfall (Marpaung et al., 2024). Similarly, in China, *Strepsicrates* spp. cause significant damage, leading to economic losses in the forestry sector (Zhenghong 2003). These examples highlight the importance of early detection and management strategies

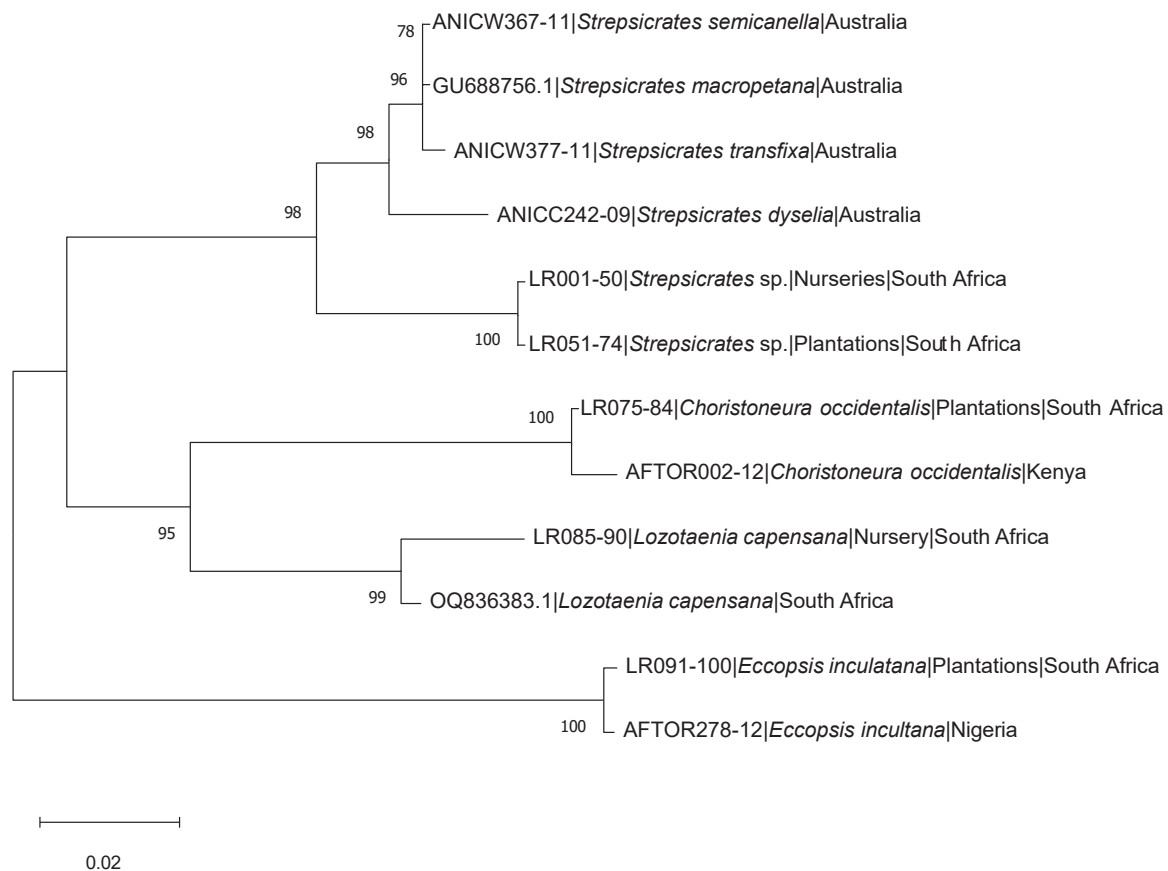


Figure 4: Phylogenetic tree based on maximum likelihood method and general time reversible model of 614 bp segment of the COI gene. The tree with the highest log likelihood (-1638.39) is shown. The percentage of trees in which the associated taxa clustered together is shown above the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2628)). Codon positions included were 1st+2nd+3rd+Noncoding. Evolutionary analyses were conducted in MEGA11.

to prevent potential outbreaks in South Africa, where the pest status of *Strepsicrates* spp. remains uncertain.

Our study marks the first record of the utilisation of eucalypts as host plants by *C. occidentalis*, *E. incultana*, and *L. capensana*. *Choristoneura occidentalis* has been reported on other hosts across Sub-Saharan Africa, including Gambia, Kenya, Tanzania, Zambia, Zimbabwe, Mozambique, and South Africa (Gilligan and Epstein 2012). The larvae of *C. occidentalis* pose a significant economic threat to *Acacia mearnsii* (Fabaceae) (Brown et al. 2014b), *Cajanus* (Fabaceae), as well as a wide range of cultivated crops, including *Allium* (Amaryllidaceae), *Coffea* (Rubiaceae), *Citrus* (Rutaceae) (Gilligan and Brown 2014) and *Pinus patula* (Pinaceae) (Austara and Jones 1971). *Eccopsis incultana* has also been reported across sub-Saharan Africa, including the Democratic Republic of Congo, Gabon, Ghana, the Gambia, Ghana, Kenya, Madagascar, Malawi, Mauritius, Namibia, Nigeria, Réunion, Sao Tome & Principe, Seychelles, Sierra Leone, South Africa, Tanzania, Zambia and Zimbabwe (Aarvik 2004; Agassiz and Aarvik 2014; De Prins and De Prins 2023). Previously reported hosts of *E. incultana* include *Vachellia tortilis* (syn. *Acacia tortilis* (Fabaceae)) in Kenya (Agassiz and Aarvik 2014), *Acacia* spp. and *Dichrostachys cinerea* (Leguminosae) in South Africa, and *Sorghum bicolor* (Poaceae) in East Africa (Natural History Museum 2023). *Lozotaenia capensana* is native to Africa and is found in The Gambia, Malawi, Mozambique, Zimbabwe, South Africa and in the South Atlantic (Austara and Jones 1971; Karisch 2003; Razowski 2015; Pinhey 1975). In South Africa, *L. capensana* has been reported on various plant species including *Chrysanthemoides monilifera* (Asteraceae), *Citrus* (Rutaceae), *Fragaria* (Rosaceae), *Lycium ferocissimum* (Solanaceae) (Taylor 1957), *Pinus radiata* (Austara and Jones 1971), *Malus domestica* (Rosaceae), *Prunus domestica* (Rosaceae), *Prunus persica* Batsch (Rosaceae), and *Vaccinium* (Ericaceae) (Prinsloo and Uys 2015).

The finding of three native leafroller species contributes to the growing list of native Lepidoptera insects adapting to non-native eucalypt hosts and other non-native trees such as pines and wattle in South Africa. Noticeable instances of native Lepidoptera insects in South Africa exhibiting this behaviour include cossid moth, *Coryphodema tristis*, on *Eucalyptus nitens* (Gebeyehu et al. 2005; Boreham 2006); pine emperor moth, *Nudaurelia cytherea* Fabr (Lepidoptera: Saturniidae) (Peringuey 1887), and pine brown tail moth, *Euproctis terminalis* (Walker) (Lepidoptera: Saturniidae) on *Pinus*; and wattle bagworm, *Kotochalia junodi* Heyl. (Lepidoptera: Psychidae) (Webb 1974; Wingfield et al. 2011) and the wattle semi-looper, *Achaea lienardi* (Boisduval) (Lepidoptera: Erebididae) on *Acacia* (Wingfield et al. 2020). Host switching of native Lepidoptera is not exclusive to South Africa; in Brazil, indigenous species like *Thyrintina arnobia* (Stoll) (Lepidoptera: Geometridae) and *Sarsina violascens* (Herrich-Schaeffer) (Lepidoptera: Lymantriidae) also defoliate eucalypt plantations (Bittencourt et al. 2003; Grosman et al. 2005; Speight and Wylie 2001). While the pest status of the newly reported native leafrollers in South Africa requires confirmation, it is noteworthy that not all native pests establishing new host associations cause significant damage or losses.

Conclusion

A number of leafroller species were found in the current study in nurseries, plantations, or both. Additional sampling is required to gather more data regarding distribution, prevalence and host associations of the leaf roller species and this should be included in surveillance activities. Further work is needed to confirm the *Strepsicrates* species present in the forest nurseries and plantations, and to determine its biology, seasonality, population dynamics, economic impact and potential management strategies.

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ORCID IDs

Privilege T Makunde — <https://orcid.org/0000-0001-6464-9472>

Bernard Slippers — <https://orcid.org/0000-0003-1491-3858>

Brett P Hurley — <https://orcid.org/0000-0002-8702-5547>

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