Phylogeny of *Holocacista capensis* (Lepidoptera: Heliozelidae) from Vineyards and Natural Forests in South Africa Inferred from Mitochondrial and Nuclear Genes

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In South Africa, the family Heliozelidae in the order Lepidoptera is restricted to four known species. The grapevine leaf miner, *Holocacista capensis*, feeds between the epidermal layers of a grapevine leaf, predominantly along the leaf margin. A final instar larva will descend from the blotch mine/gallery to attach its cocoon casing (constructed from the epidermal layers of the mined gallery) to any object below the infested leaf. Five monophyletic clades and a polyphyletic group have been identified within the Heliozelidae, using a mitochondrially encoded gene cytochrome c oxidase I (COI) and a nuclear gene, histone 3 (H3). An exploratory study of the genetic diversity within *H. capensis* populations was conducted using these genes. The phylogenetic analyses of COI indicate that *H. capensis* that are currently being collected from South Africa fall within three clades/haplotypes, of which one is well supported and contains only one species from Gauteng, and one has three specimens from two different areas in the Western Cape province, while 80% belong to haplotype 1 (H1). The current study can be used as a starting point for future DNA-based studies aimed at gaining insight into possible patterns of diversity in *H. capensis* to confirm switching from native to commercial grapevine hosts. However, more samples need to be collected from different areas in South Africa.

INTRODUCTION

The 12 monotrysian Heliozelidae genera (Lepidoptera: Adeloidea) are found on most continents, comprising 125 described species (Van Nieukerken *et al*., 2011). The family is characterised by small, drab diurnal moths, most of which have a leaf-mining larval stage (Van Nieukerken *et al*., 2011, 2012; Regier, 2015; Van Nieukerken & Geertsema, 2015; Milla *et al*., 2018). A study by Milla *et al*. (2018) presented the first global phylogenetic framework of the Heliozelidae. Using two mitochondrially encoded cytochrome c oxidase I (COI) and cytochrome c oxidase II (COII) genes and two nuclear genes, histone 3 (H3) and 28S ribosomal DNA, they identified five major monophyletic clades (*Coptodisca*, *Holocacista*, *Antispilina*, *Pseliastis* and *Hoplophanes*) and a polyphyletic group (*Antispila*) within the Heliozelidae. The relationships between the clades, however, remain unresolved due to a lack of statistical support. To resolve relationships between clades that diverged in the Late Cretaceous, it would be necessary to increase the number of nuclear genes to resolve deeper nodes by providing additional phylogenetic information. As most of the undescribed diversity (at genus and species level) occurs in the southern hemisphere, Milla *et al*. (2018) suggest that the family may have southern origins.

The African Heliozelidae fauna is restricted to four known species described in South Africa. The species are *Antispila argyrozona* Meyrick, 1918, *Holocacista capensis* Van Nieukerken & Geertsema, 2015, *Holocacista salutans* Meyrick, 1921 and *Antispilina varii* Mey, 2011. In South Africa, *H. capensis* is a multivoltine, leaf-mining pest of potential economic concern occurring on *Vitis vinifera* L. (Vitaceae) (Van Nieukerken & Geertsema, 2015). The leaf-mining larvae of *H. capensis* feed between the epidermal layers, predominantly along the leaf margin of an infested leaf (Van Nieukerken & Geertsema, 2015). A final instar larva will descend from the blotch mine/gallery to attach its cocoon casing (constructed from the epidermal layers of the mined gallery) to any object below the infested leaf.

The pest was reported for the first time in 2012 on commercial and ornamental varieties of *Vitis vinifera* in

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the surroundings of Paarl in the Western Cape province, South Africa (Van Nieukerken & Geertsema, 2015). The grapevine leaf miner is thought to have undergone a host-plant switch from the native African Vitaceae (for example, *Rhoicissus* Planch. and *Cissus* L. species) to commercial and ornamental varieties of *V. vinifera* (Van Nieukerken & Geertsema, 2015). Van Nieukerken and Geertsema (2015) also reported the grapevine leaf miner on *Rhoicissus* in the vicinity of Wilderness in the Western Cape. Steyn *et al*. (2021) identified several *H. capensis* populations on grapevine in three grape-producing regions in the Western Cape, and high population numbers were detected in the Berg River, the Hex River and the Olifants River regions. However, no wild populations (surviving on native hosts) were detected in the study. All collected individuals were only identified based on morphological features, and the identities of the collected individuals have not been confirmed using genetic analyses. As a result, the populations present within each area have not yet been confirmed as conspecific.

The aim of the current work was to combine the morphological identification of *H. capensis* with a molecular database approach of moth specimens collected from previous and current surveys. Surveys were conducted in and around the grapevine-growing regions and natural forests surrounding the Western Cape province, South Africa. This was done to gain an understanding of the genetic relationships between and diversity of leaf miner populations from diverse locations. An exploratory study of the genetic diversity of *H. capensis* populations was conducted, using mitochondrial and nuclear genes to explore whether the pest originated from local host plants, along with the genetic variation currently present within populations in the Western Cape province.

MATERIALS AND METHODS

Surveying natural forests and commercial vineyards

Holocacista capensis specimens collected in a previous study by Steyn *et al*. (2021) were used, together with specimens collected as part of a survey of the natural forests in and around the Western Cape of South Africa between November 2017 and May 2018 (Fig. 1). At least one baited yellow Delta Trap lined with a sticky pad (Chempac Pty Ltd., Paarl) was placed in each of the sampled areas. The male-biased attractant dispensers, loaded with a synthetic pheromone (Wang *et al*., 2015), were supplied by Lund University, Sweden. Ad hoc sampling was also conducted in Halfmanshof, Riebeeck Kasteel, Robertson and George, and in a variety of grape-producing areas within the Northern Cape province (Table 1).

Retrieval of male moths from sticky pads

Male moths were extracted manually from the sticky pads. A small square of the sticky pad, containing the specimen, was cut out of the trap/pad and placed in a small pool of

FIGURE 1

A graphic representation of the trapping locations to detect *Holocacista capensis* male moths (using male-biased lures) in the Western Cape, Eastern Cape and Northern Cape provinces of South Africa. Trapping efforts were focused on grape-producing areas and stands of native grapes/natural forests. Solid pins indicate areas where male moths were detected, whilst crosses are indicative of areas where sampling took place but no male moths were detected.

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eucalyptus oil (Miller *et al*., 1993). A fine paintbrush (Prime Art *Bianco* R 000) was used to ease the specimen from the sticky trap and care was taken to remove as much of the sticky trap adhesive as possible without the loss of antennae, legs or wing scales. The processed samples were stored in absolute ethanol (99%) before DNA extraction. Morphological identification of the male moth was done according to the description of Van Nieukerken and Geertsema (2015).

Molecular characterisation

Total DNA extraction was performed using a Quick-DNA Miniprep Plus Kit (Zymo Research) according to the

TABLE 1

A list of trapping locations to detect *Holocacista capensis* male moths. The locations in bold indicate where specimens were collected and where male moths were found.

Province	Region/area	Area/town	Traps placed	Vicinity	> 2 moths detected
Western Cape	Berg River	George	1	33°49’09.1”S 22°21’41.2”E	No
		Halfmanshof	**> 5**	**33°08’44.8”S 18°59’10.3”E**	**Yes**
		Piketberg	**2**	**32°58’44.8”S 18°44’55.9”E**	**Yes**
		Porterville	**1**	**33°00’38.6”S 19°00’47.6”E**	**Yes**
		Riebeeck Kasteel	**3**	**33°23’00.3”S 18°54’36.5”E**	**Yes**
		Tulbagh	**2**	**33°17’30.8”S 19°05’18.6”E**	**Yes**
		Wellington	**2**	**33°35’48.2”S 18°58’33.4”E**	**Yes**
		Wolseley	**1**	**33°24’47.7”S 19°14’06.7”E**	**Yes**
	Hex River	Ashton	1	33°49’23.2”S 19°58’41.0”E	No
		Bonnievale	2	33°51’46.1”S 19°59’12.8”E	No
		De Doorns	> 5	33°30’12.1”S 19°35’59.6”E	No
		McGregor	**1**	**33°54’11.6”S 19°52’30.7”E**	**Yes**
		Robertson	**2**	**33°50’19.0”S 19°54’52.3”E**	**Yes**
	Olifants River	Klawer	2	31°45’26.7”S 18°33’49.5”E	No
		Trawal	1	31°53’13.2”S 18°37’47.3”E	No
		Vredendal	**1**	**31°41’21.2”S 18°30’20.3”E**	**Yes**
Northern Cape	Orange River	Augrabies	3	28°42’4.58”S 20°27’25.90”E	No
		Blouputs	3	28°28’55.62”S 20° 7’13.08”E	No
		Friersdale	1	28°43’49.29”S 20°44’43.82”E	No
		Kakamas	3	28°45’42.46”S 20°33’52.29”E	No
		Kanon Eiland	1	28°39’18.45”S 21° 6’55.86”E	No
		Upington	2	28°24’24.18”S 21°19’34.60”E	No
Western Cape	Natural forests	Heidelberg	1	33°59’20.2”S 20°49’24.4”E	No
		Harkerville	1	34°03’08.2”S 23°14’08.6”E	No
		Knysna	1	33°59’30.0”S 23°07’25.0”E	No
		Nature’s Valley	2	33°58’20.1”S 23°33’02.2”E	No
		Rheenendal	2	33°54’31.5”S 22°57’49.9”E	No
		Riviersonderend	5	34°04’41.9”S 19°49’47.1”E	No
		Sedgefield	2	34°01’51.4”S 22°50’18.1”E	No
		Stellenbosch	**2**	**33°59’24.8”S 18°56’16.7”E**	**Yes**
		Suurbraak	1	34°00’07.4”S 20°37’46.6”E	No
		Wilderness	1	33°59’46.4”S 22°33’42.1”E	No
Eastern Cape		Storms River	3	33°59’51.7”S 23°57’21.7”E	No

manufacturer's instructions. The amount of DNA (ng/µl) in the final product was measured for each specimen using a spectrophotometer ND-1000 (NanoDrop Technologies) to confirm successful DNA extraction.

The amplification of four genes (COI, COII, H3 and 28S) was carried out using four different primer pairs for DNA-based identification of the specimens (Table 2). The final PCR was generated following Van Nieukerken *et al*. (2012). In short, mixtures contained 10 µl OneTaq 2 x master mix (NEB), 1 µl of the extracted genomic DNA (10 ng/µl to 30 ng/µl), 1 µl forward primer (10 µM), 1 µl reverse primer (10 µM) and 7 µl nuclease-free water. PCR protocol conditions for each of the primer pairs (COI, COII, H3 and 28S) included an initial denaturation at 94°C for 30 sec; then 35 cycles of 94°C for 30 sec, annealing between 45°C and 55°C (Table 2) for 30 sec, extension at 68°C for 1 min; and a final elongation of 68°C for 10 min. For each PCR run, a positive and negative control were included.

PCR products were run on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye (Amresco) to confirm successful amplification. PCR products were purified using the ExoSAP master mix (prepared by combining 50 µl Exonuclease I (NEB) 20 U/µl and 200 µl shrimp alkaline phosphatase (NEB) 1 U/µl). The reaction mixture was prepared by combining 10 µl of the PCR product and 2.5 µl ExoSAP master mix, and incubating this at 37°C for 30 min, followed by 95°C for 5 min.

The purified sequencing reaction products were sequenced using the BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 Nimagen, and analysed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, Thermo Scientific) with a 50 cm array, using POP7 polymer (Applied Biosystems, Thermo Scientific). Chromatogram analysis was performed using FinchTV analysis software (Geospiza). All DNA-based analyses (sequencing and PCR) were conducted at Inqaba Biotechnical Industries (Pty) Ltd., Pretoria, South Africa.

Phylogenetic analysis

The DNA sequences generated were aligned and edited in CLC Main Workbench 22.0.2 (QIAGEN Bioinformatics, Denmark). Sequences were subjected to a BLAST search (Altschul *et al*., 1997) performed in the GenBank nucleotide sequence database via the National Centre for Biotechnology Information (NCBI) (U.S. National Library of Medicine, Rockville Pike, USA), to determine the closest sequence match. To assess the phylogenetic position of the male moths collected during this survey, 76 sequences were generated and compared to other *Holocacista* spp. sequences from GenBank (see Table 3 for details). Phylogenetic analyses were conducted based on maximum likelihood (ML) using MEGA 11 (Tamura *et al*., 2021). Concatenated sequences of the COI and H3 analysis included 25 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 859 positions in the final dataset. Evolutionary analyses were conducted in MEGA 11 (Tamura *et al*., 2021).

Model testing to select the most appropriate model for each dataset was performed using MEGA 11 (Tamura *et al*., 2021). The evolutionary history of the concatenated COI and H3 sequences was inferred using the maximum composite likelihood (MCL) method and the general time reversible model (Nei & Kumar, 2000). The percentage of trees in which the associated taxa clustered together is shown next to the branches. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter = 0.3393)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. A single outgroup was selected from the Nepticulidae family, based on outgroup selections made by Milla *et al*. (2018).

The number of haplotypes, haplotype diversity (the probability of two haplotypes from the same population being different when randomly selected) and nucleotide diversity (the level of polymorphism within the population) were calculated using ARLEQUIN v.3.5.2.2 (Excoffier & Lischer, 2010).

Results

Survey and identification

The morphological identification of the male moths as *H. capensis* was based on the unique male genitalia, the wing venation and colour pattern (Van Nieukerken & Geertsema, 2015). The DNA of a total of 44 male moths was extracted

TABLE 2
Primers used for PCR amplification and sequencing of COI, COII, 28S and H3 regions, adapted from Milla *et al*. (2018).

Gene	Primer name	Annealing °C	Direction	Primer sequence (5'-3')	Reference
COI	LepF1	50	Forward	ATTCAACCAATCATAAAGATATTGG	Hebert *et al*. (2003)
	LepR1		Reverse	TAAACTTCTGGATGTCCAAAAAATCA	Hebert *et al*. (2003)
COII	COIIF	45	Forward	GGAGCATCTCCTTTAATAGAACA	Sperling *et al*. (1995)
	COIIR		Reverse	GAGACCATTACTTGCTTTCGATCATCT	Caterino & Sperling (1999)
28S	28SF	55	Forward	GAGAGTTMAASAGTACGTGAAAC	Downton & Austin (1998)
	28SR		Reverse	TCGGARGGAACCAGCTACTA	Whiting *et al*. (1997)
H3	H3HEXAF	55	Forward	ATGGCTCGTACCAAGCAGACGGC	Ogden & Whiting (2003)
	H3HEXAR		Reverse	ATATCCTTGGGCATGATGGTGAC	Ogden & Whiting (2003)

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TABLE 3
Holocacista capensis male sequences generated from forested habitats and table grape-producing regions in the surroundings of the Western Cape, South Africa, from mitochondrial (COI) and nuclear (H3) genes in this study and sequences included from GenBank.

		GENBANK accession numbers			
Specimen code	**Origin/Western Cape**	**COI**	**Bp**	**H3**	**Bp**
LS4	Riebeeck Kasteel	MT827254	608	MT846703	294
LS5	Riebeeck Kasteel	MT827255	632	MT846704	294
LS14	Riebeeck Kasteel	MT827256	641	MT846705	294
LS16	Riebeeck Kasteel	-	-	MT846706*	272
LS17	Riebeeck Kasteel	-	-	MT846707*	286
LS38	Piketberg	MT827257	632	MT846708	294
LS41	Tulbagh	MT827258	641	-	-
LS45	Tulbagh	MT827259*	490	MT846709*	281
LS46	Tulbagh	-	-	MT846710	294
LS48	Tulbagh	-	-	MT846711	294
LS53	Tulbagh	-	-	MT846712	294
LS63	Porterville	MT827260*	571	MT846713*	251
LS70	Porterville	MT827261*	206	MT846714	294
LS74	Porterville	MT827262	632	MT846715	294
LS75	Porterville	MT827263	660	MT846716	294
LS85	Vredendal	MT827264	611	MT846717*	286
LS86	Vredendal	MT827265	650	MT846718	294
LS91	Vredendal	MT827266	664	MT846719	294
LS93	Vredendal	MT827267	648	MT846720	294
LS94	Vredendal	MT827268	628	MT846721	292
LS95	Vredendal	-	-	MT846722	294
LS97	McGregor	MT827269	596	MT846723	294
LS99	McGregor	MT827270	696	MT846724	294
LS101	McGregor	MT827271	645	MT846725	294
LS102	McGregor	-	-	MT846726	294
LS103	McGregor	-	-	MT846727*	286
LS105	Robertson	-	-	MT846728	292
LS112	Robertson	MT827272	672	MT846729	292
LS113	Robertson	MT827273	648	MT846730	294
LS117	Robertson	MT827274	578	MT846731	294
LS120	Robertson	MT827275	581	MT846732	294
LS121	Robertson	MT827276	655	MT846733	294
LS132	Halfmanshof	MT827277*	379	MT846734	294
LS133	Halfmanshof	-	-	MT846735	294
LS134	Halfmanshof	MT827278*	618	MT846736	292
LS140	Halfmanshof	MT827279	647	MT846737	294
LS144	Halfmanshof	MT827280	657	MT846738	294
LS145	Wolseley	MT827281*	593	MT846739	294

TABLE 3 (CONTINUED)

		GENBANK accession numbers			
Specimen code	**Origin/Western Cape**	**COI**	**Bp**	**H3**	**Bp**
LS147	Wolseley	-	-	MT846740*	250
LS148	Wolseley	-	-	MT846741*	274
LS150	Wolseley	-	-	MT846742*	281
LS155	Jonkershoek	MT827282*	542	MT846743	294
LS156	Jonkershoek	MT827283	650	MT846744*	202
LS160	Jonkershoek	MT827284	660	MT846745	294
LS161	Paarl	MT827285	609	MT846746	294
RMNH.INS.24622	Paarl	KP697785	658	-	-
RMNH.INS.24624	Paarl	KP697788	658	-	-
RMNH.INS.29578	Paarl	KP697799	558	-	-
RMNH.INS.24260	Paarl	KP697801	685	-	-
RMNH.INS.24263	Paarl	KP697807	658	-	-
RMNH.INS.24262	Paarl	KP697812	658	-	-
RMNH.INS.24261	Paarl	KP697813	658	-	-
RMNH.INS.29586	Roodeplaat/Gauteng	KP697809	658	-	-
RMNH.INS.24260	n/a	MF118292	657	MF118477	327
RMNH.INS.24622	n/a	MF118321	657	MF118505	327
MMP.004870	n/a	MK978214	657	-	-

- = no sequence available; * = not used in analysis

and used to generate 31 COI sequences and 44 H3 sequences (Table 3). The amplification of the COII produced only three successful sequences (MT846699, MT846700, MT846701) and the 28S only one sequence (MK213721) deposited in GenBank.

Mitochondrial and nuclear gene analysis

A total of 38 *Holocacista* COI sequences were used to construct a phylogenetic tree, of which 11 were retrieved from GenBank (Table 3). Short sequences generated through this study were not used in the analysis; however, they were submitted to GenBank (Table 3). The maximum composite likelihood (MCL) in MEGA 11 (Tamura *et al*., 2021) was used to construct a phylogenetic tree (Fig. 2). The results indicated that the specimens collected in each of the areas fell within three different clades, of which the one from the specimen from Gauteng was well supported. Three specimens formed a sub-group within the phylogenetic tree, with 50% bootstrap support. The specimens were not from the same sampling site and there were representative specimens from the same areas that did not fall within this sub-group (Fig. 2).

A total of 44 H3 *Holocacista* nuclear sequences were generated in this study, and four sequences from GenBank (Table 3) were used to construct an ML phylogenetic tree. Short sequences generated were discarded, as indicated in Table 3. A final dataset of 292 positions was used. No differences were observed in any base pairs of the sequences; however, a clear difference was found between different *H. rivillei* and *H. varii* (Fig. 3). Clusters found in the phylogenetic analysis were not supported by the tree that was generated as a result, as it showed no base pair differences (Fig. 3). It can be seen from Fig. 4 that, using the 26 concatenated sequences of COI and H3, two haplotypes were well supported.

COI and H3 genetic diversity

Based on the COI sequences for 20 *H. capensis* specimens, the haplotype (0.353 ± 0.123) and nucleotide (0.003 ± 0.002) diversity were low. Eight polymorphic sites and three haplotypes were recorded (Table 4). Haplotype 1 (H1) was the most frequently encountered and accounted for approximately 80% of all the specimens used in the COI phylogenetic assessment. Haplotype 2 (H2) was recorded from McGregor and Riebeeck Kasteel in addition to H1, whilst haplotype 3 (H3) occurred only in Vredendal in addition to H1. H1 was limited to all other areas (Piketberg, Tulbagh, Porterville, Robertson, Halfmanshof, Wolseley, Jonkershoek and Paarl) (Fig. 2).

DISCUSSION

The *H. capensis* samples collected by Steyn *et al*. (2021) and in the current study were identified as *H. capensis* based on commonly noted morphological traits (Steyn *et al*., 2020,

2021) in all the detected populations within each of the regions and natural environments in this study. This confirmation permitted the preliminary investigation of the genetic relationships and diversity between existing *H. capensis* leaf-mining populations within these environments.

The low genetic diversity, based on haplotype/nucleotide diversity and the presence of a few, and seemingly closely related, haplotypes was unexpected, considering that *H. capensis* is thought to be a native pest. Although one can only speculate (due to the scale of the sampling efforts adopted in this study), this finding is in contrast with expectations of a theoretically native lepidopteran that would not have been exposed to typical bottleneck effects of alien and invasive insect pests (Nei *et al*., 1975; Roderick & Navajas, 2003), as seen in the case of *Cameraria ohridella* Deschka & Dimić (Gracillaridae) (Lees *et al*., 2011). Unless, of course, a recent

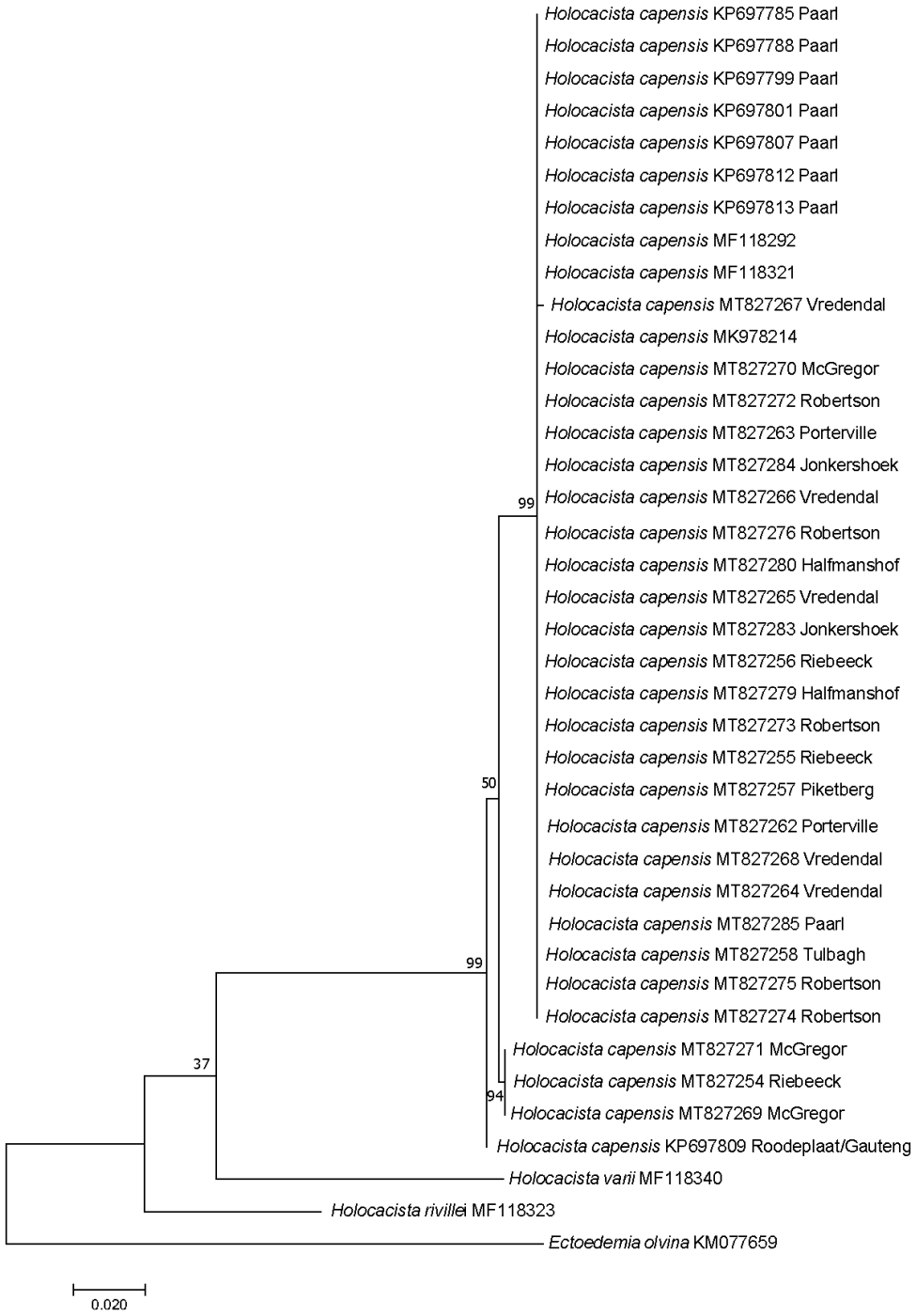

FIGURE 2

Maximum likelihood phylogenetic tree (highest log likelihood = -790.53) showing the relationship of *Holocacista capensis* with other species based on 39 COI nucleotide sequences, with a total of 578 positions in the final dataset. Numbers at the nodes represent bootstrap support (50% or more, 1 000 replicates). All positions containing gaps and missing data were eliminated.

Evolutionary analyses were conducted in MEGA11 (Tamura *et al*., 2021). *Ectoedemia olvina* was selected as an outgroup.

change in host-plant preferences has led to the establishment of a new leaf miner strain that is not affected by the attractant that has been developed using individuals collected from commercial grapevines, and the native populations remain undetected. To gain more clarity on this issue, more rigorous sampling efforts need to be adopted in more diverse habitats in future studies.

Ball and Armstrong (2006) concluded that, in the case of lymantriid lepidopteran species, DNA barcoding using COI is promising, especially for taxa that are well defined at the species level. It is also true, however, that COI is a maternally inherited mitochondrial gene and thus cannot be used as a detection tool for discerning hybridisation events. In this case, sequencing the nuclear H3 gene did

FIGURE 3

Maximum likelihood phylogenetic tree showing the relationship between 38 *Holocacista* species collected from different areas based on the nuclear H3 gene, with 292 positions in the final dataset. Numbers at the nodes represent bootstrap support (50% or more, 1 000 replicates). All positions containing gaps and missing data were eliminated. *Ectoedemia olvina* was used as the outgroup.

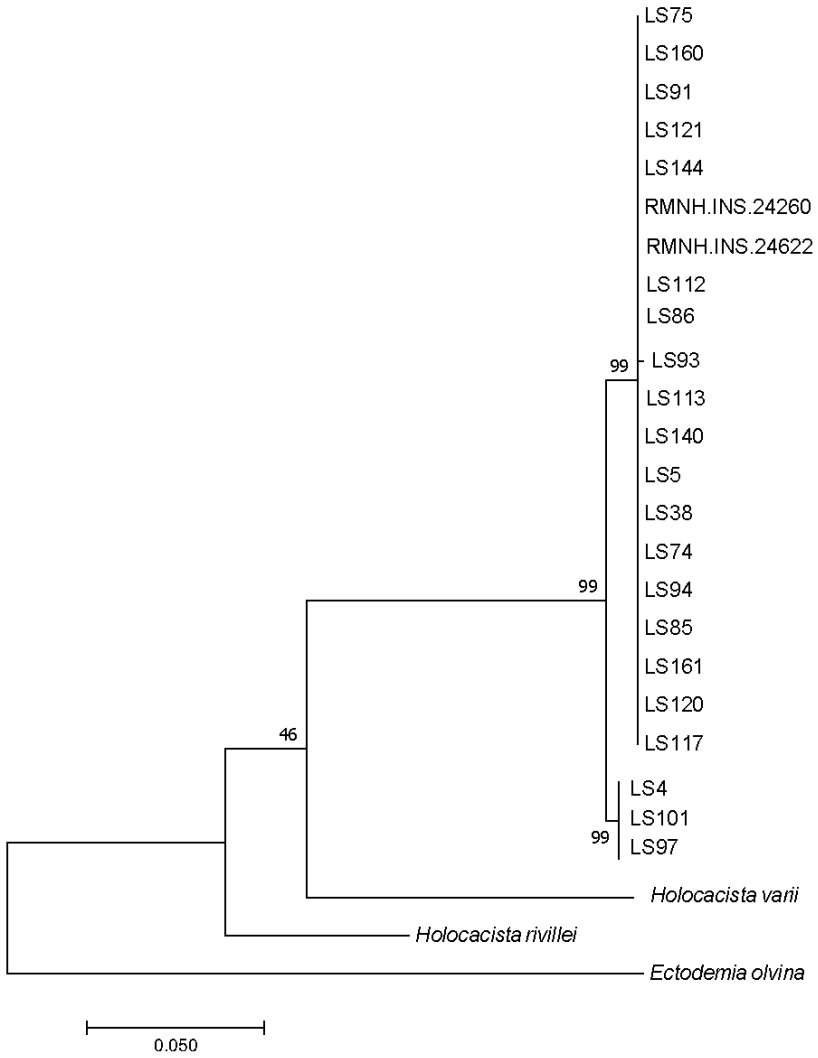

FIGURE 4

Maximum likelihood phylogenetic tree from concatenated sequences of a mitochondrially encoded gene cytochrome c oxidase I (COI) and a nuclear gene, histone 3 (H3), showing the relationships between *Holocacista capensis* specimens. The tree was based on 26 concatenated sequences with 859 bp in the final dataset. The percentage of trees in which the associated taxa clustered together is shown next to the branches. All positions containing gaps and missing data were eliminated. *Ectoedemia olvina* was selected as the outgroup.

TABLE 4

The eight polymorphic sites of the three COI haplotypes of *Holocacista capensis*.

Haplotype	Site							
	2	**8**	**81**	**89**	**290**	**329**	**549**	**562**
H1	C	G	G	T	G	G	A	C
H2	T	A	.	C	A	A	G	T
H3	.	.	A	.	.	.	.	.

not validate the presence of hybridised individuals and does not reflect the same sub-grouping phenomenon noted in the COI phylogeny. However, it is possible that nuclear mitochondrial pseudogenes (numts) (which are essentially non-functional copies of mtDNA within the nucleus, which become specifically problematic when a short fragment of the mitochondrial COI gene is amplified) are responsible for the phenomenon noted in this study (Song *et al*., 2008).

The survey of the natural forests of the Western Cape and surroundings yielded disappointing results. *Holocacista capensis* males were only collected from one of the natural forests in Jonkershoek (Stellenbosch) and it is likely that the

moths were present in higher numbers in the surroundings of the potentially infested vineyards (and thus present in Jonkershoek) on the foothills of Stellenbosch Mountain.

Interestingly, the genetic variation in *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) from its native range (South America) and those of the invaded countries of the Mediterranean mirrors the genetic homogeneity of *H. capensis* found in the current study (Cifuentes *et al*., 2011). Cifuentes *et al*. (2011) attributed this to the founder effects experienced by invading populations of *T. absoluta* as an invasive species. Assefa *et al*. (2013) recorded similar findings for *Eldana saccharina* Walker (Lepidoptera: Pyralidae) in invaded regions, although considerably high genetic diversity was recorded between populations in the regions of West Africa, Ethiopia and South Africa. These studies raise questions regarding the origin of *H. capensis*, which can only be answered through more rigorous sampling of native populations on natural hosts and the analysis of other higher resolution genetic markers.

Even though conclusions based on the genetic relationships and diversity of *H. capensis* cannot be drawn, the current study identified limited genetic variation in infested, commercial landscapes, and a lack of regional genetic variation accommodates ubiquitous control efforts for which chemical intervention is necessary. It is evident that more surveys and investigations are required to obtain more representative specimens from other provinces in South Africa. Future research efforts should include more rigorous sampling in natural and invaded landscapes, and would ideally include a survey of the infested grapevines in Gauteng province, South Africa, where the leaf miner has been detected in the past. The current study can be used as a starting point for future studies focused on establishing the relationships between *H. capensis* in their native hosts and the commercial hosts to which they have switched.

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