INTRODUCTION

The introduction and successful establishment of non-native populations of threatened species is becoming increasingly common. Today, this occurs frequently as an impact of illegal wildlife trade, but past introductions of common species that have subsequently undergone declines in their native range are also increasingly being recognized (Gibson & Yong, 2017). These non-native populations are steadily being acknowledged for their potential to be used for conservation programs in the form of translocations back into native ranges (Garzón-Machado, del-Arco-Aguilar, & Pérez-de-Paz, 2012; Marchetti & Engstrom, 2016). These translocations can lead to the genetic rescue of native populations, which can alleviate negative genetic impacts associated with small, isolated populations, such as...
low genetic diversity, increased genetic drift, and inbreeding depression (Weeks et al., 2011). However, using non-native populations of threatened species for genetic rescue is likely to come with challenges; founder effects are commonly encountered in introduced populations, presenting a loss of genetic diversity from the native population, and an increase in genetic drift (Frankham et al., 1999). Nevertheless, recent investigations of non-native populations of a number of threatened species show only minor differences in genetic variation between introduced and native populations, highlighting that these perceived negative genetic impacts associated with introduced populations are not always present, especially if insufficient time has passed following introductions for genetic drift and divergence to occur, or if multiple introductions of the same species have occurred (Collins, Freeman, & Snow, 2008; Vörös, Mitchell, Waldman, Goldstien, & Gemmell, 2008). These factors can vary across introduced populations/species owing to life-history traits and time since introduction, and therefore, the need to evaluate the suitability of threatened, non-native populations for their potential as candidates for translocations needs to be considered on a case-by-case basis.

In Australia, several introduced species, particularly from the Order Artiodactyla, have since become threatened with extinction in their native range; the IUCN lists both sambar (Rusa unicolor) and Javan rusa deer (Rusa timorensis) as vulnerable, while banteng (Bos javanicus) and hog deer (Axis porcinus) are listed as endangered in their native range. The use of the Australian banteng population for conservation has been explored in the past (Bradshaw, Isagi, Kaneko, Bowman, & Brook, 2006; Bradshaw et al., 2007); however, little attention has been given to the hog deer population present in Victoria. The hog deer is native to South-East Asia and is present in Pakistan, India, Nepal, and Bangladesh, as well as small, introduced populations in Sri Lanka and the United States (Timmins et al., 2015). Declines throughout their native range have been associated with overhunting, predominantly for meat, trophies, and velvet antler used in traditional medicine, and conversion of their preferred habitat of tall floodplain grasslands for agriculture and commercial development (Timmins et al., 2015). The largest non-native population of hog deer exists in Australia, where a stable, continuous population occurs throughout the Gippsland region in Victoria (Scroggie, Forsyth, & Brumley, 2012). Hunting of hog deer is restricted to April every year, and only one male and one female may be harvested per season per hunter, in part because the population is thought to be important for the long-term conservation of the species. Assessing the potential for the hog deer in Victoria to be used for conservation efforts is therefore not only important for the declining populations within the native range, but also for the management of the deer in Victoria. Negative impacts to native flora and fauna due to deer damage are well known, and hog deer can therefore be managed more effectively to mitigate these impacts if their conservation worth is effectively evaluated (Davis et al., 2016; Davis, Coulson, & Forsyth, 2008; Davis, Forsyth, & Coulson, 2010).

There are a number of considerations to be made when assessing the Victorian hog deer’s suitability for translocations as part of conservation programs. Firstly, it is important to identify which subspecies has established in Victoria. The Acclimatisation Society of Victoria brought hog deer from South-East Asia to Victoria in the 1860s, primarily sent from ports in Sri Lanka and India (Mayze & Moore, 1990). The Sri Lanka population itself is thought to be comprised of introduced hog deer of unknown origin (Timmins et al., 2015). Furthermore, following these introductions to Australia, a new subspecies of hog deer was described in South-East Asia, the Indochinese hog deer (Axis porcinus annamiticus; Heude 1888). Today, this subspecies is considered critically endangered and occurs in isolated populations in Cambodia and Thailand; however, in the past its distribution was more widespread (Brook, Nask, & Channa, 2015; Maxwell, Nareth, Kong, Timmins, & Duckworth, 2006). Hunters in Victoria have also previously reported two distinct “forms” of hog deer found throughout Gippsland, with one form described as being smaller with a stockier build (Bentley, 1978).

The second consideration when evaluating the Victorian hog deer population for translocations is the possibility that either past or contemporary hybridization has occurred. Hybridization is prolific within the family Cervidae, and hybrid zones have been recorded in the genera Cervus (Lowe & Gardiner, 1975; McDevitt et al., 2009; Moore & Littlejohn, 1989; Senn & Pemberton, 2009), Odocoli neus (Ballinger, Blankenship, Bickham, & Carr, 1992; Carr, Ballinger, Derr, Blankenship, & Bickham, 1986; Cathey, Bickham, & Patton, 1998), and most recently in Rusa (Martins, Schmidt, Lenz, Wilting, & Fickel, 2018). While hybridization between species within the Axis genus has not been reported in the wild, there have been cases of chital (Axis axis) and hog deer hybrid offspring being born in captivity, with animals from the two species needing to be separated due to their proclivity to interbreed (Gray, 1972; Mayze & Moore, 1990; McMaster, 1871). Chital have been released in Victoria in the past, including in areas where hog deer are now known to occur; however, these chital populations have since become locally extirpated (Forsyth, Stamatier, & Woodford, 2016). Many species of deer were housed together in Royal Park (now the Royal Melbourne Zoological Gardens) prior to release (“A Few Hours in the Zoological and Acclimatisation Society’s Grounds” 1873; “The Naturalist”, 1873), so it is possible that hog deer and chital were housed in captivity together prior to their liberation. Additionally, previous research has shown that while native hog deer and chital mitochondrial genomes share a 94.65% identity, the mitochondrial genomes of Victorian hog deer and chital show a greater degree of similarity which may indicate hybridization; however, this was detected in only four samples from a managed island population in Victoria, and so may not be representative of the entire population (Hassanin et al., 2012; Hill, Linacre, Toop, Murphy, & Strugnell, 2017). There are also unconfirmed reports that another species from the Axis genus, the Bawean hog deer (Axis kuhlii), was introduced to Victoria and possibly released; however, there is some debate that the species introduced was actually the Javan rusa (Bentley, 1978; Mayze & Moore, 1990). Today, the hog deer range in Victoria overlaps with both sambar and fallow deer (Dama dama; Forsyth, Stamatier, & Woodford, 2015; Forsyth et al., 2016). While hybridization across two different genera of deer...
is likely to be rare, hybridization between fallow and hog deer has been recorded in captivity in the past, although it is unknown how long the offspring survived, or if they were fertile (Gray, 1972).

The final consideration necessary to evaluate the suitability of hog deer for conservation efforts is to estimate the genetic diversity present within the Victorian population. When choosing individuals to use for translocations, it has been suggested that capturing >95% of the genetic variation present in the source population is necessary in order to offset the negative genetic impacts present in the receiving population (Weeks et al., 2011). The founding population

**FIGURE 1** Maps showing the collection sites for samples used for sequencing analysis in this study: (a) chital (Axis axis) sampling locations in Queensland, Australia; (b) hog deer (Axis porcinus) sampling locations in Victoria, Australia; and (c) Indochinese hog deer (Axis porcinus annamiticus) sampling location in Koh Kong Province, Cambodia. Green circles indicate sites where sequences for all five genes were obtained, and purple circles indicate sites where only the D-loop was sequenced.
of hog deer in Victoria was only comprised of 15 individuals (nine females, two males, and four of unknown sex); however, the population has thrived since being released, and a continuous breeding population is present across a 2,336 km² range (Forsyth et al., 2016; Mayze & Moore, 1990). In order to capture as much genetic diversity as possible, it is crucial to sample individuals throughout the entire range in Victoria to determine how much variation is present in the population, and if particular sites contain greater diversity and are therefore more suitable for translocation.

Genetic analyses of mitochondrial and nuclear DNA are able to address the suitability of the Victorian population of hog deer as a potential source population for conservation efforts of the species. Mitochondrial markers, and the nuclear markers alpha-lactalbumin (αLalb) and protein kinase C iota I (PRKCI), have been used in the past to investigate the phylogeny and genetic diversity of various deer species (Ludt, Schroeder, Rottmann, & Kuehn, 2004; Vernesi et al., 2004) and have been shown to be effective at delineating species (Hassanin & Douzery, 2005). These markers will be utilized in this study to first establish which species or subspecies of hog deer was introduced to Australia, and then to assess the genetic diversity and any occurrence of hybridization within this population in order to ascertain the value of the Victorian population as a source for genetic rescue within the native range.

2 | METHODS

2.1 | Sampling and DNA extraction

A total of 78 liver and tongue samples were collected from wild, free-ranging hog deer (presumed *Axis porcinus*) during the hunting seasons from 2015 to 2017, in Victoria (VIC), Australia (Figure 1). Museum Victoria provided a further two voucher samples of Australian hog deer, collected during a cull at Wilsons Promontory National Park in 2015 (Museum No. Z52238 and Z52239). Additionally, samples were collected from two deer species also belonging to the genus *Axis*. These included 35 tissue samples from the wild, free-ranging chital (Axis axis), collected in Queensland (QLD), and two skin samples from the Indochinese hog deer (*Axis porcinus annamiticus*), collected from Cambodia (KHM) in the Koh Kong Province (Figure 1). Extractions of tissue samples were carried out using a DNeasy Blood and Tissue Kit (Qiagen), following the manufacturer’s instructions, and DNA was quantified using a Qubit 2.0 Fluorometer (Invitrogen).

| Gene region | Primer name | Primer sequence (5′–3′) | n (Hog deer, Chital, A. p. annamiticus) | Fragment length | Reference |
|-------------|-------------|-------------------------|--------------------------------------|----------------|-----------|
| COI         | LCO1490     | GGTCAACAAATCATAAAAGATATTG| 22, 5, 1                            | 567 bp         | Folmer et al. (1994) |
|             | HCO2198     | TAAACTTCCGGTGACGAAAAAATCA  |                                       |                |           |
| Cyt b       | L14724      | CGAAGCTTATGAAAAAACATCGTTG | 22, 5, 1                            | 387 bp         | Kocher et al. (1989) |
|             | H15149      | AAACCTGCCCATCCAGATATTTGTCTCA |                                      |                |           |
| D-loop      | FD15378     | CCTAAGACTCAAGGAAGAAGGCTA  | 80, 35, 2                           | 576 bp         | This study |
|             | R16130      | GATGCAGTTAAGTCCAGCTACATT |                                       |                |           |
| αLalb       | αLalbF      | ATCTGTAACATCTCTGTGTA     | 22, 5, 1                            | 382 bp         | Hassanin and Douzery (2003) |
|             | αLalbR      | TCAGTAAAGRCATCATCCAG     |                                       |                |           |
| PRKCI       | U26         | TATGCTAAAAGTGCTGGTGG     | 22, 5, –                            | 412 bp         | Ropiquet and Hassanin (2005) |
|             | L748         | CTGTACCAGCTAATCCT        |                                       |                |           |

2.2 | PCR amplification

Three mitochondrial markers and two nuclear markers were used in this study. Universal markers for the mitochondrial genes, cytochrome b (Cyt b) and cytochrome oxidase subunit I (COI), were utilized, as well as custom-made D-loop primers (Branicki, Kupiec, & Pawlowski, 2003; Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994; Kocher et al., 1989; Table 1). Nuclear regions of intron 2 of the α-lactalbumin gene (αLalb) and the intron of protein kinase C iota (PRKCI) were also sequenced (Hassanin & Douzery, 2003; Ropiquet & Hassanin, 2005). Twenty-two samples of VIC hog deer and five samples of QLD chital were sequenced for the above-described genes. All regions were also sequenced for one *Axis porcinus annamiticus* sample, except the PRKCI gene which failed to amplify. The samples of hog deer selected for sequencing of all five gene regions were chosen to represent the spatial distribution of the hog deer population in Victoria. A further 58 samples of hog deer, 30 samples of chital, and one additional sample of *A. p. annamiticus* were sequenced for the D-loop region to assess the genetic diversity of the populations. This region was chosen to further investigate genetic diversity as the mitochondrial control region, where the D-loop is located, is considered to be the most polymorphic section of the mitochondrial genome, and several studies have utilized the D-loop to assess genetic diversity in several deer species in the past (Hu, Fang, & Wan, 2006; Moritz, Dowling, & Brown, 1987; Pérez-Espona et al., 2009; Skog et al., 2009).

Sequence PCRs were carried out in 25 μl reactions, consisting of 12.5 μl of MyTaq™ Red Mix (Bioline), 0.5 μl (10 μM) of forward primer, 0.5 μl (10 μM) of reverse primer, 1.5 μl of MgCl₂, 8 μl of H₂O, and 2 μl of template DNA, ranging from 3.69 to 317 ng/μl. Cycling
conditions were the same for each gene region, with an initial denaturation at 95°C for 15 min, 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min, followed by a final extension of 72°C for 5 min. PCR products were visualized on a 1% agarose gel and sent to Macrogen Inc. (South Korea) for sequencing.

2.3 Data analysis

2.3.1 Phylogenetics

Sequences were aligned using the Geneious alignment method in Geneious 9.0.5 (Kearse et al., 2012). Additional sequences of hog deer and chital were downloaded from GenBank to include in the dataset (Table 2). Further sequences of all deer species that have been introduced to Australia, including those that did not become established, were also included in the dataset where available (Table 2). Sequences of moose (Alces alces) were used as an outgroup for phylogenetic analyses. These sequences were trimmed and aligned with the hog deer, chital, and Indochinese hog deer sequences. Mitochondrial genes were concatenated for analyses, while the nuclear genes αLalb and PRKCI were analyzed separately. Alignments were run in JModelTest 2.1.7 to determine the best-fit model for each alignment, using the Akaike information criterion (Akaike, 1974; Darriba, Taboada, Doallo, & Posada, 2012). Best-fit models were GTR + I + G for the concatenated mitochondrial alignment, TrN + G for the αLalb gene, and F81 + G for the PRKCI gene. Bayesian phylogenetic analyses were run in MrBayes 3.2.6 to calculate posterior probabilities, which were run for 1,000,000 generations, after a burn-in period of 100,000, and sampling trees every 200 generations (Huelsenbeck & Ronquist, 2001). This analysis was performed twice for every gene, to ensure convergence toward the same likelihood score. Maximum likelihood phylogenetic trees were created using the program PHYML, using the same models described previously, with 1,000 bootstrap replicates (Guindon et al., 2010). The program PAUP 4.0 was used to calculate genetic distances for the concatenated mitochondrial tree, using the model identified in JModelTest 2.1.7 (Swofford, 1999).

| Latin name                  | Common name            | Mitochondrial | αLalb   | PRKCI   |
|-----------------------------|------------------------|----------------|---------|---------|
| Axis axis                   | Chital                 | JN632599<sup>a</sup> | DQ379348<sup>c</sup> | DQ379329<sup>c</sup> |
| Axis kuhlii                 | Bawean hog deer        | –              | –       | –       |
| Axis porcinus               | Hog deer               | JN632600<sup>a</sup> | DQ379349<sup>c</sup> | DQ379367<sup>c</sup> |
| Capreolus capreolus         | Roe deer               | JN632610<sup>a</sup> | –       | DQ122021<sup>d</sup> | DQ365692<sup>c</sup> |
| Cervus canadensis songaricus*| Wapiti/Elk             | KJ025072<sup>b</sup> | –       | –       |
| Cervus elaphus              | Red deer               | NC007704       | –       | DQ379345<sup>c</sup> |
| Cervus nippon               | Sika deer              | AB211429       | DQ379352<sup>c</sup> | DQ379332<sup>c</sup> |
| Dama dama                   | Fallow deer            | JN632629<sup>a</sup> | DQ379356<sup>c</sup> | DQ379335<sup>c</sup> |
| Hydropotes inermis          | Chinese water deer     | JN632649<sup>a</sup> | –       | DQ379340<sup>c</sup> |
| Moschus moschiferus*        | Musk deer              | JN632662<sup>a</sup> | –       | DQ365693<sup>c</sup> |
| Muntiacus muntjak           | Indian muntjac         | NC004563       | –       | –       |
| Odocoileus hemionus         | Mule deer              | JN632670<sup>a</sup> | –       | DQ379345<sup>c</sup> |
| Odocoileus virginianus      | White-tailed deer      | JN632672<sup>a</sup> | –       | DQ379346<sup>c</sup> |
| Rangifer tarandus           | Reindeer               | NC007703       | –       | DQ379346<sup>c</sup> |
| Rucervus duvauceli           | Barasingha             | JN632696<sup>a</sup> | –       | DQ379331<sup>c</sup> |
| Rucervus eldii              | Eld's deer             | JN632697<sup>a</sup> | –       | DQ379353<sup>c</sup> |
| Rusa timorensis             | Javan rusa deer        | JN632699<sup>a</sup> | –       | DQ379333<sup>c</sup> |
| Rusa unicolor               | Sambar deer            | NC008414       | –       | DQ379343<sup>c</sup> |

Note: These species were all historically introduced to Australia (present-day species in Australia in bold), as reported by Moriarty (2004). Sequences could not be found for C. canadensis and Moschus sibiricus; so, closely related species were used as a substitute (asterisk).

<sup>a</sup>Hassanin et al. (2012).
<sup>b</sup>Li, Ba, and Yang (2016).
<sup>c</sup>Gilbert et al. (2006).
<sup>d</sup>Hassanin and Douzery (2003).
<sup>e</sup>Matthee, Burzlafl, Taylor, and Davis (2001).
TABLE 3  D-loop sequences of chital (Axis axis), A. porcinus, and A. p. annamiticus taken from GenBank that were combined with the main dataset

| Species                  | Accession no.          | Location     | Reference                                       |
|--------------------------|------------------------|--------------|-------------------------------------------------|
| Axis axis                | JN596132–JN596143      | India        | Direct submission                               |
|                          | JN596145–JN596148      |              |                                                 |
|                          | JN596150–JN596151      |              |                                                 |
|                          | JN596153–JN596156      |              |                                                 |
|                          | JN632599               | Captive      | Hassanin et al. (2012)                          |
| Axis porcinus            | EF491198–EF491199      | Thailand     | Direct submission                               |
|                          | EF491201–EF491202      |              |                                                 |
|                          | EU870592               | India        | Direct submission                               |
|                          | JN632600               | Captive      | Hassanin et al. (2012)                          |
| Axis porcinus annamiticus| MH392156–MH392168      | India        | Gupta et al. (2018)                             |
|                          | KM881614–KM881625      | Thailand     | Direct submission                               |

FIGURE 2  Bayesian phylogenetic tree based on 1,530 bp concatenated mtDNA regions of COI, Cyt b, and the D-loop. Bayesian posterior probability values are reported above each node; maximum likelihood bootstrap values are reported below the nodes. GenBank accession numbers are written next to species names. Blue text indicates hog deer species; purple text indicates deer species currently established in Australia; and red text indicates deer species historically introduced to Australia. Sample sizes are specified where multiple samples shared the same haplotype. Alces alces was used as an outgroup.

2.3.2  Population genetics

Sequences of the D-loop region were trimmed to 576 bp and aligned using the Geneious alignment method in Geneious 9.0.5 (Kearse et al., 2012). Measures of haplotype diversity and nucleotide diversity were calculated for the VIC hog deer and QLD chital using DNAsp 5.10.1 (Librado & Rozas, 2009). The neutrality tests Tajima’s D and Fu’s Fs were calculated for each population in the program Arlequin 3.5 (Excoffier & Lischer, 2010; Fu, 1997; Tajima, 1989). Samples collected in this study were then trimmed to 372 bp to align with native samples of chital, A. porcinus, and A. p. annamiticus downloaded from GenBank in order to create a haplotype network (Table 3). Samples of hog deer taken from GenBank were assigned to either A. porcinus or A. p. annamiticus based on the species names reported in GenBank. A median-joining haplotype network was generated using the program PopART (Leigh & Bryant, 2015). Populations of native...
chital, native *A. porcinus*, and native *A. p. annamiticus* (including two *A. p. annamiticus* samples collected in this study) were also analyzed for the genetic diversity and neutrality indices described above.

### 3 | RESULTS

#### 3.1 | Phylogenetics

All unique sequences generated in this study for each species have been deposited in GenBank (accession no. MN226858–MN226880). A 567-base-pair (bp) region of the COI gene, a 387-bp region of the Cyt b gene, and a 576-bp fragment of the D-loop region were successfully amplified in each of the 22 Victorian hog deer, five Queensland chital, and one Cambodian Indochinese hog deer samples, to create a concatenated 1,530-bp mitochondrial fragment. Topologies from the Bayesian analysis of the mitochondrial data show that both VIC hog deer and QLD chital fall within a single clade, along with one sample of *Axis axis* available in GenBank (accession no. JN632600), again with highly supported PP and BS values. Genetic distances calculated between the VIC hog deer and the QLD and GenBank chital reveal a distance of 0.003–0.005 (Table 4), considerably lower than observed for interspecific genetic distances between other species in the *Axis* genus (Figure 3). Interspecific genetic distances between other species in this genus ranged from 0.03 to 0.11, with the lowest value representing the difference between *Axis porcinus* and the subspecies *Axis porcinus annamiticus*.

Figure 2). This clade was highly supported, with posterior probability (PP) and bootstrap (BS) values being 1 and 100, respectively. All samples of QLD chital were shown to share the same haplotype at the three mitochondrial genes, while samples of VIC hog deer also comprised a single haplotype for the three mitochondrial genes. The sample of Indochinese hog deer was shown to form a separate clade with the sample of *Axis porcinus* available in GenBank (JN632600), again with highly supported PP and BS values.

#### Table 4

|                    | Hog Deer VIC | *A. porcinus* | *A. p. annamiticus* | *A. axis* QLD | *A. axis* |
|--------------------|--------------|---------------|---------------------|---------------|-----------|
| *A. porcinus*      | 0.09         | 0.11          | 0.03                |               |           |
| *A. p. annamiticus*| 0.11         | 0.06          | 0.11                | 0.03          |           |
| *A. axis* QLD      | 0.003        | 0.09          | 0.11                | 0.005         |           |
| *A. axis*          | 0.005        | 0.09          | 0.11                | 0.003         |           |

#### Figure 3

MtDNA pairwise distances (GTR + I + G) within hog deer and chital species (intraspecific), between separate species in the *Axis* genus (interspecific), and between species belonging to different genera (intergeneric), which includes samples of *Dama dama* and *Rucervus duvaucelli*. Data points highlighted in purple represent pairwise comparisons between Victorian hog deer and *Axis porcinus* and *Axis axis* samples.
**Figure 4** Bayesian phylogenetic tree based on 382 bp of the αLab gene. Bayesian posterior probability values are reported above each node; maximum likelihood bootstrap values are reported below the nodes. GenBank accession numbers are written next to species names. Blue text indicates hog deer species; purple text indicates deer species currently established in Australia; and red text indicates deer species historically introduced to Australia. Sample sizes are specified where multiple samples shared the same haplotype. *Alces alces* was used as an outgroup.

**Figure 5** Maximum likelihood phylogenetic tree based on 412 bp of the PRKCI gene. Bayesian posterior probability values are reported above each node; maximum likelihood bootstrap values are reported below the nodes. GenBank accession numbers are written next to species names. Blue text indicates hog deer species; purple text indicates deer species currently established in Australia; and red text indicates deer species historically introduced to Australia. Sample sizes are specified where multiple samples shared the same haplotype. *Alces alces* was used as an outgroup.
when compared to intraspecific genetic distance within the Axis genus (range from 0 to 0.003).

A 382 bp of the αLab region and a 412-bp region of the PRKCI gene were successfully amplified from each sample of this study. Phylogenetic analyses of the αLab gene revealed that a polytomy was formed with the Victorian hog deer, the Queensland chital samples, Axis porcinus annamiticus, and an Axis porcinus sample taken from GenBank (Figure 4). This clade was highly supported by both PP and BS values (1 and 92, respectively). A total of five haplotypes were identified in the Victorian hog deer population, and 11 of these samples shared the same haplotype as Axis porcinus DQ379349. The chital samples from Queensland appear to be more distinct from the Victorian hog deer samples while still belonging to the same group; however, these samples did not cluster with the GenBank sample of Axis axis. The Axis axis DQ379348 sample is shown to form a clade with Axis porcinus DQ379349 in Gilbert, Ropiquet, and Hassanin (2006); however, in the present study Axis axis DQ379348 formed a clade with the outgroup (Alces alces) and was shown to be identical, suggesting that this sample has been mislabelled in GenBank and is likely to be Alces alces.

Topologies of the PRKCI Bayesian analyses again reveal that the Victorian hog deer samples are within the same group as the Axis axis samples, forming a polytomy with Axis axis DQ379329 and Axis porcinus DQ379367 (Figure 5). All samples within this polytomy are highly supported, with PP and BS values of 1 and 82, respectively. Two haplotypes are present in the Victorian hog deer, with the most common of the two being shared with one Queensland chital sample and a GenBank Axis axis sample. The second VIC hog deer haplotype, the remaining Queensland chital samples, and the GenBank Axis porcinus sample all form separate, distinct branches within this group.

### 3.1.1 Population genetics

A 576-bp fragment of the D-loop was successfully sequenced in 35 chital from QLD, 80 hog deer from VIC, and two samples of A. p. annamiticus from Cambodia. A single haplotype was identified in the hog deer population, indicating that the population is monomorphic for this gene. The QLD chital population comprises two distinct haplotypes, with a haplotype diversity of 0.461 (±0.07 SD) and a nucleotide diversity of 0.0023 (±0.0035 SD) (Table 5). In comparison, much greater diversity was detected in the native chital and hog deer populations sourced from GenBank. The number of observed haplotypes ranged from 12 in the native A. p. annamiticus samples to 17 in the A. porcinus group. The number of polymorphic sites was also much higher, with 21 observed in the native chital, and 26 observed in both the A. porcinus and A. p. annamiticus samples. Haplotype diversity ranged from 0.913 (±0.02 SD) in the native chital to 0.988 (±0.02 SD) in the native A. porcinus, while nucleotide diversity ranged from 0.017 (±0.0015 SD) to 0.023 (±0.0019 SD) in the native populations (chital and A. porcinus, respectively). Neutrality tests were not significant in any of the populations.

The median-joining haplotype network created with the combined chital and hog deer data generated in this study and taken from GenBank shows four distinct groups within the data (Figure 6). Only two haplotypes are present in the Australian populations, both of which are found in the broader chital group. One of these haplotypes is shared between the VIC hog deer and QLD chital, with all hog deer individuals and 10 chital samples containing this haplotype. The haplotypes detected in Australian samples of hog deer and chital were not observed in the native chital samples; however, for both Australian haplotypes, only one bp difference is observed between these samples and the nearest related native chital sample. The native hog deer samples separated into three groups: an A. porcinus group, an A. p. annamiticus group, and a mixed group comprising samples from both subspecies. Only two samples were present in the A. p. annamiticus group, which were both sequenced in the present study. All other samples belonging to A. p. annamiticus as reported in GenBank were clustered into the mixed group with samples identified as A. porcinus, with four haplotypes being shared between the two subspecies.

### Table 5

Genetic diversity measurements based on a fragment of the D‐loop for the hog deer population in Victoria, the chital population in Queensland, and samples from the native range of chital, Axis porcinus, and Axis porcinus annamiticus.

|                  | Native A. porcinus | Native A. p. annamiticus | Hog deer VIC | A. axis QLD | Native A. axis |
|------------------|--------------------|--------------------------|--------------|-------------|---------------|
| n                | 19                 | 14                       | 80           | 35          | 23            |
| N_h              | 17                 | 12                       | 1            | 2           | 14            |
| S                | 26                 | 26                       | 0            | 4           | 21            |
| h (±SD)          | 0.988 (0.02)       | 0.978 (0.04)             | 0            | 0.461 (0.07)| 0.913 (0.04) |
| Χ (±SD)          | 0.023 (0.0019)     | 0.019 (0.0062)           | 0            | 0.0023 (0.0004)| 0.017 (0.0015)|
| Tajima’s D       | 0.290              | 0.560                    | –            | 0.880       | –0.466        |
| P_TD             | 0.43               | 0.31                     | –            | 0.82        | 0.39          |
| Fu’s Fs          | -1.267             | 2.151                    | –            | 2.593       | 0.830         |
| P_FS             | 0.26               | 0.82                     | –            | 0.89        | 0.66          |

Note: n, sample size; N_h, number of haplotypes; S, number of polymorphic sites; h, haplotype diversity; Χ, nucleotide diversity; SD, standard deviation; P_TD, probability of Tajima’s D; P_FS, probability of Fu’s Fs.
The mitochondrial and nuclear data presented here portray an interesting insight into the recent genetic history of hog deer in Victoria, Australia. Due to the discovery of chital haplotypes in the mitochondrial DNA of hog deer in Victoria, it is not possible to identify which species/subspecies of hog deer was initially introduced using traditional barcoding methods. Results from the nuclear gene region $\alpha$LaLb indicate that these haplotypes are more closely related to *Axis porcinus* rather than *Axis porcinus annamiticus*, which may suggest that the species introduced to Australia was the Indian hog deer *A. porcinus*. However, given the low levels of resolution at this nuclear marker, additional analysis using more variable nuclear STRs or SNPs is needed to firmly conclude which hog deer species was released in Victoria.

The mitochondrial data showed that hog deer in Victoria possess haplotypes that are most closely allied with the chital, a species that historically was released and established a population in Victoria but has since become locally extirpated (Forsyth et al., 2016). This may be a result of incomplete lineage sorting; however, it is more likely that hybridization has occurred between these two species. The time of divergence between chital and *Axis porcinus* occurred during the Pliocene, approximately 2.6 Mya, and a number of mitochondrial haplotypes exclusive to each species have been detected, as seen in the haplotype network presented in this study (Gilbert et al., 2006; Gupta et al., 2018; Hassanin et al., 2012). The presence of these species-specific haplotypes suggests that incomplete lineage sorting is unlikely to be a factor influencing the mitochondrial results reported here. Hog deer and chital are also morphologically distinct; mature hog deer have a stocky build, reach 60–70 cm in shoulder height, and have a reddish-brown coat color with a dark dorsal stripe across the back of the neck and spine, with light-colored spots along either side of the dorsal stripe. Chital are larger, finer in build with a shoulder height of 70–90 cm, and a reddish-brown coat color covered in distinct white spots. These differences, along with the absence of a wild chital population in Victoria, highlight that the results reported here are unlikely to be due to misidentification based on morphological appearance. Additionally, hybrids between captive hog deer and chital have been recorded previously. These have typically arisen from the mating of a male hog deer with a female chital, as seen in the present study with the presence of chital haplotypes in the maternally inherited mitochondrial genome. Similar unidirectional hybridization has been reported in red deer and sika deer hybrids, with genetic contributions from female red deer and male sika deer being reported a majority of the time when analyzed throughout Ireland and the UK (Smith, Carden, Coad, Birkitt, & Pemberton, 2014; Smith et al., 2018). In both cases, hybridization occurs between the female of the larger deer species (chital and red deer) and the male of the smaller deer species (hog deer and sika deer), and with males generally being the larger sex this hybridization pattern may be reflective of the phenotypic limitations that the reciprocal cross would present. However, this may be less of a consideration between hog deer and chital as overall sizes are relatively similar, and crosses have been reported to occur both ways (Gray, 1972). Hybrids between hog deer and chital also appear to favor hog deer-like phenotypes. McMaster (1871) describes hybrids as having similar behavioral characteristics as hog deer, and with darker fur and fainter spots than chital, while Gray (1972) reported a hybrid that resembled hog deer in “head, face, and horns,” with a white-spotted coat resembling chital. It is also possible that backcrossing to a parental species has occurred following the hybridization event, which may further explain the hog deer appearance seen in the current population. Furthermore,
the presence of a shared nuclear haplotype between Victorian hog deer and *Axis porcinus* at the αLalb gene provides further support for hybridization between the two species of deer.

This study is the first to report on long-term persistence of chital and hog deer hybrids in a wild setting. It is important to note, however, that it is unknown when the initial hybridization occurred; hybridization may have arisen in the wild in either the native range or after release of both chital and hog deer in Wilsons Prom in the 1860s or may have occurred in captivity prior to release. The scenario presented here is also somewhat unusual; traditionally, hybridization occurs when the distribution of two genetically distinct populations shares overlapping ranges, mates, and produces viable offspring, with the offspring forming a hybrid zone where overlap occurs (Shurtliff, 2013). However, no pure stock of chital is present within Victoria as the population became locally extinct in the 1920s, and no pure hog deer were identified during the course of this study. This would suggest that either both parental species have been essentially bred out of existence over generations, or only hybrids were ever released into the wild. As the original parental species are no longer present, further assessment of hybridization within Victoria, particularly detecting backcrossing to either species, is difficult as samples of parental species that contributed to the hybridization are needed for more in-depth analysis.

The continued survival of the hybrid population in Victoria over many generations without the presence of either parental species demonstrates that hybrids between chital and hog deer are fertile. The chromosome numbers differ in these two species (chital 2n = 66, hog deer 2n = 68; Khongcharoensuk et al., 2017; Pinthong et al., 2017); however, this is not unique to hog deer and chital, with other species known to hybridize in the family Cervidae also comprising different chromosome numbers (Bonnet-Garnier, Claro, Thevenon, Gautier, & Hayes, 2003; Gustavsson & Sundt, 1968). Robertsonian translocations of chromosomes, whereby the fusion of whole arms of two acrocentric chromosomes occurs, are common in cervids (Bonnet-Garnier et al., 2003; Huang, Chi, Nie, Wang, & Yang, 2006), and it is likely that the prevalence of these chromosome translocations has assisted in the production of fertile hybrids where chromosome numbers are different. Robertsonian translocations have been detected in red deer and sika deer hybrids (Herzog & Harrington, 1991), and it is feasible that further investigation of hybrid hog deer and chital through karyotyping will reveal similar mutations that have made fertile hybrids possible.

The mitochondrial D-loop region was discovered to be monomorphic within the Victorian hog deer population, suggesting that the diversity at this region of the mitochondrial genome is very low. Similar findings have been reported for other species introduced to Australia belonging to the Order Artiodactyla; mitochondrial analysis of Banteng revealed that this species was monomorphic at one mitochondrial gene and two nuclear genes (Bradshaw et al., 2006), and analysis of the D-loop of Australian populations of dromedary camels (*Camelus dromedarius*) revealed only 13 haplotypes, which was considered low by the authors as the founder size of dromedary camels was <5,000 individuals, and their population size is now considered to be greater than 1 million animals (Spencer et al., 2012). Many more D-loop haplotypes were observed in the native chital, *A. porcinus* and *A. p. annamiticus* populations, which is unsurprising as the Victorian hog deer population has undergone a bottleneck following introduction to Australia, and the lack of variation may leave the population vulnerable to stochastic events. However, as genetic diversity was only measured using a single mitochondrial gene, the addition of more samples and faster evolving nuclear markers will likely elucidate a better understanding of the genetic diversity within this population. The D-loop results also show that the two samples of *A. p. annamiticus* sequenced for this study appear to be genetically distinct from all other reported *A. p. annamiticus* samples present in the haplotype network. These samples are the first *A. p. annamiticus* individuals to be sequenced from Cambodia, and all other samples present in GenBank are reported to have been sourced from Thailand, where populations became extinct in the 1960s but have since been reintroduced from unknown stock (Brook et al., 2015; Humphrey & Bain, 1990; Maxwell et al., 2006). These distinct *annamiticus* haplotype differences between Cambodia and Thailand warrant further research to ascertain whether the Cambodian *A. p. annamiticus* haplotype is distributed elsewhere and is in need of conservation intervention.

Despite low reported genetic diversity which is suggested to negatively impact populations, the initial population size of hog deer has expanded considerably since their release in Victoria. This may be explained by the enemy release hypothesis, whereby an introduced species becomes abundant and a successful invader as their population sizes are no longer affected by their native predators or pathogens (Keane & Crawley, 2002). In their native range, hog deer are an important prey item for many species, including leopard (*Panthera pardus fusca*), clouded leopard (*Neofelis nebulosa*), Burmese python (*Python bivittatus*), Bengal tiger (*Panthera tigris tigris*), and dhole (*Cuon alpinus*), and so, liberation from these predators is likely to positively impact abundance of the hog deer in Victoria (Dhungel & O’Gara, 1991; Grassman, Tewes, Silvy, & Kreetiyutantorn, 2005; Prasainai, Sukmasuang, Bhumpakphan, Wajjwalku, & Nittaya, 2012; Wegge, Odden, Pokharel, & Storaas, 2009). Alternatively, there may have been some genetic fitness associated with the population, particularly immediately after hybridization. Heterosis, or “hybrid vigor,” may have made initial establishment of the hog deer in Victoria much easier than if pure stock alone had been introduced. Hybridization introduces many novel alleles into the population with which natural selection can act upon, thereby increasing overall fitness (Weeks et al., 2011; Whiteley, Fitzpatrick, Funk, & Tallmon, 2015). Hybrid vigor has been implicated in the successful introductions of many plant species (Durand et al., 2002; Moody & Les, 2002) and is now being recognized as advantageous in several animal species as well (Drake, 2006; Facon, Jarne, Pointier, & David, 2005). However, as a small founding population was established and no additional hog deer were introduced to the main population following initial release, these potential genetic benefits are likely no longer affecting the persistence of the Victorian population.
Although genetic variation is reported to be low at the mitochondrial regions in the Victorian hog deer population, it may still be worthwhile to use this population as a source for genetic rescue. Due to the discovery of hybridization with chital, translocation should be restricted to areas where both species are present in the northern regions of India. It is currently unknown how prolific hybridization is between hog deer and chital in their native range, but considering that the two species share overlapping ranges, the existence of a hybrid zone is probable. Alternatively, if natural hybrid zones are not detected in the native range after extensive study, this may give further credence to the idea that hybrids between the two species only occur in captivity and, as such, would narrow down the possibilities of where hybridization most likely occurred in the Victorian population. Often, the most significant concern cited when attempting genetic rescue is the possibility of introducing outbreeding depression in the population, whereby the offspring of parents with distinct genetic differences show a decrease in overall fitness, as they are no longer well adapted to their current environment. Currently, there is a shift away from the belief that translocation of distinct populations will lead to outbreeding depression and "genetic swamping" of locally adapted alleles (Frankham, 2015; Weeks et al., 2011). The introduction of eight Texas panthers (Puma concolor stanleyana) to the declining population of Florida panthers (Puma concolor coryi) was shown to positively affect the survival rate in hybrid panther offspring, while birth rates and pup survival increased after crossing distinct lineages of Mexican wolves (Canis lupus baileyi), demonstrating that some fitness advantages can be observed when crossing distinct populations (Fredrickson, Siminski, Woolf, & Hedrick, 2007; Pimm, Dollar, & Bass, 2006). The long-term persistence of the hybrid population in Victoria suggests that outbreeding depression was not a significant factor that hindered population expansion when these crosses first occurred.

Recent genetic analysis has revealed that although hog deer have declined in their native range, this is not necessarily reflected in their genetic diversity, particularly in India (Gupta et al., 2018). Gupta et al. (2018) report moderately high levels of genetic diversity within the major Indian populations at both mitochondrial and microsatellite markers; however, lower levels are reported within Manipur, a trend which the authors attribute to fragmentation of this population. It is important to note that in this study, Gupta et al. (2018) have conducted a considerable amount of their sampling within the most abundant remnant populations of A. porcinus, which is reflective in their results and not necessarily representative of all extant populations of hog deer in India. Multiple mitochondrial haplotypes of hog deer are also reported in Pakistan, of which very little information is known about their current abundance and distribution (Abbas et al., 2017; Timmins et al., 2015). While the occurrence of greater diversity is evident in extant populations with higher levels of abundance, a number of native populations of hog deer are numbered in the hundreds, and local extinctions have been reported in up to 35 localities in India alone, which indicates that a number of populations may still be in need of intervention (Timmins et al., 2015). Additionally, it may be more beneficial to use individuals that have been sourced from Victoria rather than the native range, so as not to reduce abundance in the few relatively large remaining populations in India. Ultimately, while high diversity is currently present in the most abundant remaining populations in the native range, the Victorian population still likely represents an important safeguard to any future native declines.

Ultimately, translocations of hog deer require knowledge of both the source population and the receiver population. High priority should now be given to further sampling of hog deer (both A. porcinus and A. p. annamiticus) throughout their native range owing to their continued decrease in abundance; sites from India and Thailand have been sequenced in the past, but hog deer are known to occur in a number of countries including Pakistan, Nepal, Bangladesh, Bhutan, and Cambodia with little to no genetic assessment of these populations (Timmins et al., 2015). Future research within the native range should focus on the connectivity of hog deer across the native landscape and identifying the subspecies boundary between A. porcinus and A. p. annamiticus, with an overarching goal of promoting genetic diversity and effective management of hog deer across South-East Asia. Hog deer have also previously been reported in China, Myanmar, Viet Nam, and Laos; however, it may be locally extinct in these areas, so monitoring in habitats where hog deer have previously occurred in these countries is needed to firmly establish whether local extirpation has occurred. Additionally, an investigation into the possibility of a natural hybrid zone between hog deer and chital in India is necessary, as this will likely dictate whether translocation of Victorian hog deer is suitable for genetic rescue of the species in India. Further analysis of the Victorian population of hog deer is also warranted; this study was unable to examine the genetic diversity of Victorian hog deer in-depth as the D-loop region of the mitochondria within this population was monomorphic. Moreover, the nuclear markers chosen for this study were shown to provide low discrimination power when comparing hog deer and chital sequences. The inclusion of polymorphic nuclear STR or SNP analysis would likely address these lingering questions and could also be used for monitoring hog deer populations pre- and post-translocation, to understand the long-term effects of using Victorian hog deer hybrids as a source for genetic rescue within the hog deer native range.

ACKNOWLEDGMENTS

This project was jointly funded by the RFA grant “Securing Food, Water and the Environment” (La Trobe University) and the Victorian Game Management Authority. We would like to thank hunters who provided access to samples, and to Victorian Hog Deer Checking Station operators, Parks Victoria, and Museums Victoria for assisting with the collection of hog deer samples; Stewart McGlashan for the collection of chital samples, and Sarah Brook for the collection of Indochinese hog deer samples. The authors would also like to thank Jessica Presnell for assistance with genetic diversity analyses and Katherine Harrisson, Nikeisha Caruana, and James O’Dwyer for feedback on the manuscript. Additional thanks are also given to the
two anonymous reviewers who provided valuable feedback to the submitted manuscript.

**CONFLICT OF INTERESTS**

The authors declare that they have no conflict of interest.

**AUTHOR CONTRIBUTIONS**

EH, AL, ST, NM, and JS contributed to the design of the study; ST and EH arranged collection of Australian samples; EH arranged collection of Cambodian samples; EH performed the laboratory procedures; EH conducted the analyses; EH led the writing of the manuscript with input from NM and JS; AL, ST, NM, and JS provided feedback and revisions of the manuscript. All authors contributed equally to the drafts and gave final approval for publication.

**DATA AVAILABILITY STATEMENT**

Data are available in GenBank under accession no. MN226858–MN226880.

**ORCID**

Erin Hill [https://orcid.org/0000-0002-7642-696X](https://orcid.org/0000-0002-7642-696X)

Jan Strugnell [https://orcid.org/0000-0003-2994-637X](https://orcid.org/0000-0003-2994-637X)

**REFERENCES**

Abbas, G., Nadeem, A., Babar, M. E., Hussain, T., Tahir, M. S., Shehzad, W., & Javed, M. (2017). Molecular phylogeny and diversity analysis of hog deer (Axis porcinus) in Pakistan. *Pakistan Journal of Zoology*, 49(5), 1701–1712.

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19(6), 716–723. [https://doi.org/10.1109/TAC.1974.1100705](https://doi.org/10.1109/TAC.1974.1100705)

Ballarat Courier (1873). A few hours in the zoological and acclimatisation society's grounds. *Ballarat Courier* (Vic.: 1869–1882; 1914–1918), p. 2. Retrieved from http://nla.gov.au/nla.news-article192277420

Ballinger, S. W., Blankenship, L. H., & Bickham, J. W. (1992). Allozyme and mitochondrial DNA analysis of a hybrid zone between white-tailed deer and mule deer (Odocoileus) in west Texas. *Biochemical Genetics*, 30(1), 1–11. [https://doi.org/10.1007/BF00554423](https://doi.org/10.1007/BF00554423)

Bentley, A. (1978). An introduction to the deer of Australia: With special reference to Victoria. *Melbourne, Vic.: The Koetong Trust Service Fund.*

Bonnert-Garnier, A., Claro, F., Thevenon, S., Gautier, M., & Hayes, H. (2003). Identification by R-banding and FISH of chromosome arms involved in Robertsonian translocations in several deer species. *Chromosome Research*, 11(7), 649–663.

Bradshaw, C. J. A., Isagi, Y., Kaneko, S., Bowman, D. M. J. S., & Brook, B. W. (2006). Conservation Value of Non-Native Banteng in Northern Australia. *Conservation Biology*, 20(4), 1306–1311. [https://doi.org/10.1111/j.1523-1739.2006.00428.x](https://doi.org/10.1111/j.1523-1739.2006.00428.x)

Bradshaw, C. J., Isagi, Y., Kaneko, S., Brook, B. W., Bowman, D. M., & Frankham, R. (2007). Low genetic diversity in the bottlenecked population of endangered non-native banteng in northern Australia. *Molecular Ecology*, 16(14), 2998–3008. [https://doi.org/10.1111/j.1365-294X.2007.03365.x](https://doi.org/10.1111/j.1365-294X.2007.03365.x)

Branicki, W., Kupiec, T., & Pawlowski, R. (2003). Validation of cytochrome b sequence analysis as a method of species identification. *Journal of Forensic Sciences*, 28(1), 1–5

Brook, S. M., Nask, C., & Channa, P. (2015). Indochinese hog deer Axis porcinus annamiticus on the brink of extinction. *Deer Specialist Group. ISSN 2312-4644.*

Carr, S. M., Ballinger, S. W., Derr, J. N., Blankenship, L. H., & Bickham, J. W. (1986). Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west Texas. *Proceedings of the National Academy of Sciences of the United States of America*, 83, 9576–9580. [https://doi.org/10.1073/pnas.83.24.9576](https://doi.org/10.1073/pnas.83.24.9576)

Cathey, J. C., Bickham, J. W., & Patton, J. C. (1998). Interspecific hybridization and nonconcordant evolutionary history of maternal and paternal lineages in North American deer. *Evolution*, 52(4), 1224–1229. [https://doi.org/10.1111/j.1558-5646.1998.tb01850.x](https://doi.org/10.1111/j.1558-5646.1998.tb01850.x)

Collins, T. M., Freeman, B., & Snow, S. (2008). Final report: Genetic characterization of populations of the nonindigenous Burmese python in Everglades National Park. Final report for the South Florida Water Management District. Miami, FL: Department of Biological Sciences, Florida International University.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). *jModelTest2:* More models, new heuristics and parallel computing. *Nature Methods*, 9(8), 772.

Davis, N. E., Bennett, A., Forsyth, D. M., Bowman, D. M. J. S., Lefroy, E. C., Wood, S. W., ... Johnson, C. N. (2016). A systematic review of the impacts and management of introduced deer (family Cervidae) in Australia. *Wildlife Research*, 43(6), 515–532. [https://doi.org/10.1071/WR16148](https://doi.org/10.1071/WR16148)

Davis, N. E., Coulson, G., & Forsyth, D. M. (2008). Diets of native and introduced mammalian herbivores in shrub-encroached grassy woodland, south-eastern Australia. *Wildlife Research*, 35(7), 684–694. [https://doi.org/10.1071/WR08042](https://doi.org/10.1071/WR08042)

Davis, N. E., Forsyth, D. M., & Coulson, G. (2010). Facilitative interactions between an exotic mammal and native and exotic plants: Hog deer (Axis porcinus) as seed dispersers in south-eastern Australia. *Biological Invasions*, 12(5), 1079–1092. [https://doi.org/10.1007/s10530-009-9525-1](https://doi.org/10.1007/s10530-009-9525-1)

Dhunge, S. K., & O’Gara, B. W. (1991). Ecology of the hog deer in Royal Chitwan National Park, Nepal. *Wildlife Monographs*, 119, 3–40.

Drake, J. M. (2006). Heterosis, the catapault effect and establishment success of a colonizing bird. *Biological Letters*, 2(2), 304–307. [https://doi.org/10.1098/rsbl.2006.0459](https://doi.org/10.1098/rsbl.2006.0459)

Durand, C., Manuel, M., Boudouresque, C., Meinesz, A., Verlaque, M., & Le Parco, Y. (2002). Molecular data suggest a hybrid origin for the invasive Cauderpa racemosa (Caulerpales, Chlorophyta) in the Mediterranean Sea. *Journal of Evolutionary Biology*, 15(1), 122–133. [https://doi.org/10.1046/j.1420-9101.2002.00370.x](https://doi.org/10.1046/j.1420-9101.2002.00370.x)

Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3), 564–567.

Facon, B., Jarne, P., Pointier, J. P., & David, P. (2005). Hybridization and invasiveness in the freshwater snail *Melanoïdes tuberculata*: Hybrid vigour is more important than increase in genetic variance. *Journal of Evolutionary Biology*, 18(3), 524–535. [https://doi.org/10.1111/j.1420-9101.2005.00887.x](https://doi.org/10.1111/j.1420-9101.2005.00887.x)

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine and Freshwater Research*, 35(5), 294–299.

Forsyth, D. M., Stamation, K., & Woodford, L. (2015). *Distributions of Sambar deer, Rusa deer and Sika deer in Victoria.* Heidelberg, Vic.: Arthur Rylah Institute for Environmental Research.
Forsyth, D. M., Stamation, K., & Woodford, L. (2016). Distributions of Fallow deer, Red deer, Hog deer and Chital deer in Victoria. Heidelberg, Vic.: Arthur Rylah Institute for Environmental Research. Unpublished Client Report for the Biosecurity Branch.

Frankham, R. (2015). Genetic rescue of small inbred populations: Meta-analysis reveals large and consistent benefits of gene flow. Molecular Ecology, 24(11), 2610–2618. https://doi.org/10.1111/mec.13139

Frankham, R., Lees, K., Montgomery, M. E., England, P. R., Lowe, E. H., & Briscoe, D. A. (1999). Do population size bottlenecks reduce evolutionary potential? Animal Conservation, 2, 255–260. https://doi.org/10.1017/S136794309900058X

Fredrickson, R. J., Siminski, P., Woolf, M., & Hedrick, P. W. (2007). Genetic rescue and inbreeding depression in Mexican wolves. Proceedings of the Royal Society B: Biological Sciences, 274(1623), 2365–2371.

Fu, Y.-X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics, 147(2), 915–925.

Garzón-Machado, V., del Arco-Aguilar, M. J., & Pérez-de Paz, P. L. (2012). Threat or threatened species? A paradox in conservation biology. Journal for Nature Conservation, 20(4), 228–230. https://doi.org/10.1016/j.jnc.2012.03.001

Gibson, L., & Yong, D. L. (2017). Saving two birds with one stone: Solving the quandary of introduced, threatened species. A paradox in conservation biology. Journal for Nature Conservation, 20(4), 228–230. https://doi.org/10.1016/j.jnc.2012.03.001

Gray, A. P. (1972). Mammalian hybrids: A check-list with bibliography. Slough, UK: Commonwealth Agricultural Bureau.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Systematic Biology, 59(3), 307–321. https://doi.org/10.1093/sysbio/sys010

Gupta, S. K., Kumar, A., Angom, S., Singh, B., Ghazi, M. G. U., Tuboi, C., & Hussain, S. A. (2018). Genetic analysis of endangered hog deer (Axis porcinus) reveals two distinct lineages from the Indian subcontinent. Scientific Reports, 8(1), 16308.

Gustavsson, I., & Sundt, C. O. (1968). Karyotypes in five species of deer (Cervidae) from Victoria, Australia, using MiSeq sequencing. Mitochondrial DNA Part B. Resources, 2(2), 453–454.

Huang, L., Chi, J., Nie, W., Wang, J., & Yang, F. (2006). Phylogenomics of several deer species revealed by comparative chromosome painting with Chinese muntjac paints. Genetics, 127(1–3), 25–33. https://doi.org/10.1090/1070-0005-2449-5

Hulsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics, 17(8), 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Humphrey, S. R. B., & Bain, J. R. (1990). Endangered animals of Thailand. Gainesville, FL: Sandhill Crane Press.

Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution, 17(4), 164–170. https://doi.org/10.1016/S0169-5347(02)02499-0

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Khongcharoensuk, H., Tanomtong, A., Patawang, I., Supanuam, P., Sornnok, S., & Pinthong, K. (2017). Karyotype and idiogram of the Axis Deer (Axis axis, Cervidae) by conventional staining, G-, high-resolution GTG-, and Ag-NOR-banding techniques. Cytologia, 82(1), 91–98. https://doi.org/10.1508/cytologia.82.91

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablana, F. X., & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the United States of America, 86, 6196–6200. https://doi.org/10.1073/pnas.86.16.6196

Leigh, J. W., & Bryant, D. (2015). popart: Full-feature software for haplotype network construction. Methods in Ecology and Evolution, 6(9), 1110–1116.

Li, Y., Ba, H., & Yang, F. (2016). Complete mitochondrial genome of Cervus elaphus sangurosus (Cetartiodactyla: Cervinae) and a phylogenetic analysis with related species. Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis, 27(1), 620–621. https://doi.org/10.1080/19401736.2014.908373

Librado, P., & Rozas, J. (2009). DNA SP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11), 1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Lowe, V. P. W., & Gardiner, A. S. (1975). Hybridization between red deer (Cervus elaphus) and sika deer (Cervus nippon) with particular reference to stocks in N.W. England. Journal of Zoology, 177, 553–566. https://doi.org/10.1111/j.1469-7998.1975.tb02259.x

Ludt, C. J., Schroeder, W., Rottmann, O., & Kuehn, R. (2004). Mitochondrial DNA phylogeography of red deer (Cervus elaphus). Molecular Phylogenetics and Evolution, 31(3), 1064–1083. https://doi.org/10.1016/j.ympev.2003.10.003

Marchetti, M. P., & Engstrom, T. (2016). The conservation paradox of endangered and invasive species. Conservation Biology, 30(2), 434–437. https://doi.org/10.1111/cobi.12642

Martins, R. F., Schmidt, A., Lenz, D., Wilting, A., & Fickel, J. (2018). Human-mediated introduction of introgressed deer across Wallace's Land barrier. Mammalian hybrids: A check-list with bibliography. Cytologia, 82(1), 91–98. https://doi.org/10.1508/cytologia.82.91

Hill, E., Linacre, A. M., Toop, S., Murphy, N. P., & Strugnell, J. M. (2017). The complete mitochondrial genome of Axis porcinus (Mammalia: Cervidae) from Victoria, Australia, using MiSeq sequencing. Mitochondrial DNA Part B. Resources, 2(2), 453–454.

Hu, J., Fang, S.-G., & Wan, Q.-H. (2006). Genetic diversity of Chinese water deer (Hydroplotes inermis inermis): Implications for conservation, Biochemical Genetics, 44(3–4), 156–167. https://doi.org/10.1007/s10528-006-9020-7

Marchetti, M. P., & Engstrom, T. (2016). The conservation paradox of endangered and invasive species. Conservation Biology, 30(2), 434–437. https://doi.org/10.1111/cobi.12642

Maxwell, A., Nareth, C., Kong, D., Timmins, R., & Duckworth, J. (2006). Hog deer (Axis porcinus) confirmed in the wild in eastern Cambodia. Natural History Bulletin of the Siam Society, 54, 227–237.

Mayze, R. J., & Moore, G. (1990). The hog deer. Croydon, Vic.: Australian Deer Research Foundation.
McDevitt, A. D., Edwards, C. J., O’Toole, P., O’Sullivan, P., O’Reilly, C., & Carden, R. F. (2009). Genetic structure of, and hybridization between, red (Cervus elaphus) and sika (Cervus nippon) deer in Ireland. Mammalian Biology, 74(4), 263–273. https://doi.org/10.1016/j.mambio.2009.03.015

McMaster, A. C. (1871). Notes on Jerdon’s mammals of India. Chennai, India: Higginbotham and Co.

Moody, M. L., & Les, D. H. (2002). Evidence of hybridity in invasive watermilfoil (Myriophyllum) populations. Proceedings of the National Academy of Sciences of the United States of America, 99(23), 14867–14871. https://doi.org/10.1073/pnas.172391499

Moore, G. H., & Littlejohn, R. P. (1989). Hybridisation of farmed wapiti (Cervus elaphus manitobensis) and red deer (Cervus elaphus). New Zealand Journal of Zoology, 16(2), 191–198. https://doi.org/10.1080/03014223.1989.10422568

Morarly, A. (2004). The liberation, distribution, abundance and management of wild deer in Australia. Wildlife Research, 31(3), 291–299. https://doi.org/10.1071/WR02100

Moritz, C., Dowling, T., & Brown, W. (1987). Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. Annual Review of Ecology and Systematics, 18(1), 269–292. https://doi.org/10.1146/annurev.es.18.110187.001413

Pérez-Espona, S., Pérez-Barbería, F., Goodall-Copestake, W., Jiggins, C., Gordon, I., & Pemberton, J. (2009). Genetic diversity and population structure of Scottish Highland red deer (Cervus elaphus) populations: A mitochondrial survey. Heredity, 102(2), 199. https://doi.org/10.1038/hdy.2008.111

Pimm, S. L., & Lollar, D., & Bass, O. L. Jr (2006). The genetic rescue of the Florida panther. Animal Conservation, 9(2), 115–122. https://doi.org/10.1017/j.1469-1795.2005.00010.x

Pinthong, K., Tanomtong, A., Khongcharoenkus, H., Chaiphech, S., Rattanayuvakorn, S., & Supanum, P. (2017). Karyotype and idiogram of Indian Hog Deer (Hyelaphus porcinus) by Conventional Staining, G,TG-, high-resolution and Ag-NOR banding techniques. Cytologia, 82(3), 227-233. https://doi.org/10.1508/cytologia.82.227

Prasani, K., Sukamasung, R., Bhumpakphan, N., Waijwalku, W., & Nittaya, K. (2012). Population characteristics and viability of the introduced hog deer (Axis porcinus) in Phu Khieo Wildlife Sanctuary, Thailand. Songklanakarin Journal of Science & Technology, 34(3), 263-271.

Ropiquet, A., & Hassanin, A. (2005). Molecular evidence for the polyphyly of the genus Hemitragus (Mammalia, Bovidae). Molecular Phylogenetics and Evolution, 36(1), 154-168. https://doi.org/10.1016/j.ympev.2005.01.002

Scroggie, M. P., Forsyth, D. M., & Brumley, A. R. (2012). Analyses of Victorian hog deer (Axis porcinus) checking station data: demographics, body condition and time of harvest. Arthur Rylah Institute of Environmental Research Technical Report Series, No. 230.

Senn, H. V., & Pemberton, J. M. (2009). Variable extent of hybridization in invasive sika (Cervus nippon) and native red deer (C. elaphus) in a small geographical area. Molecular Ecology, 18(5), 862–876. https://doi.org/10.1111/j.1365-294X.2008.04051.x

Shurtliff, Q. R. (2013). Mammalian hybrid zones: A review. Mammal Review, 43(1), 1–21. https://doi.org/10.1111/j.1365-2970.2011.00205.x

Skog, A., Zachos, F., Rueness, E., Feulner, P., Mysterud, A., Langvatn, R., ... Hartl, G. (2009). Phylogeography of red deer (Cervus elaphus) in Europe. Journal of Biogeography, 36(1), 66–77.

Smith, S. L., Carden, R. F., Coad, B., Birkitt, T., & Pemberton, J. M. (2014). A survey of the hybridisation status of Cervus deer species on the island of Ireland. Conservation Genetics, 15(4), 823–835. https://doi.org/10.1007/s10592-014-0582-3

Smith, S. L., Senn, H. V., Pérez-Espona, S., Wyman, M. T., Heap, E., & Pemberton, J. M. (2018). Introggression of exotic Cervus (nippon and canadensis) into wild deer (Cervus elaphus) populations in Scotland and the English Lake District. Ecology and Evolution, 8(4), 2122–2124.

Spencer, P. B. S., Giustiniano, D., Hampton, J. O., Gee, P., Burrows, N., Rose, K., ... Woolnough, A. P. (2012). Identification and management of a single large population of wild dromedary camels. Journal of Wildlife Management, 76(6), 1254–1263. https://doi.org/10.1002/jwmg.381

Swofford, D. L. (1999). PAUP* 4.0. Sunderland, MA: Sinauer.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 123(3), 585–595.

Timmer, R., Duckworth, J. W., Samba Kumar, N., Anwarul Islam, M., Sagar Baral, H., Long, B., & Maxwell, A. (2015). Axis porcinus. The IUCN Red List of Threatened Species. Retrieved from http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T417844A22157664.en. Downloaded on 15 May 2018.

Vernesi, C., Pecchioli, E., Caramelli, D., Tiedemann, R., Randi, E., & Bertorello, G. (2002). The genetic structure of natural and reintroduced roe deer (Capreolus capreolus) populations in the Alps and central Italy, with reference to the mitochondrial DNA phylogeography of Europe. Molecular Ecology, 11(8), 1285–1297. https://doi.org/10.1046/j.1365-294X.2002.01534.x

Vörös, J., Mitchell, A., Waldman, B., Goldstien, S., & Gemmell, N. J. (2008). Crossing the Tasman Sea: Inferring the introduction history of Litoria aurea and Litoria raniformis (Anura: Hylidae) from Australia into New Zealand. Austral Ecology, 33(5), 623–629.

Weeks, A. R., Sgro, C. M., Young, A. G., Frankham, R., Mitchell, N. J., Miller, K. A., ... Hoffmann, A. A. (2011). Assessing the benefits and risks of translocations in changing environments: A genetic perspective. Evolutionary Applications, 4(6), 709–725. https://doi.org/10.1111/j.1752-4571.2011.00192.x

Wegge, P., Odden, M., Pokharel, C. P., & Storaas, T. (2009). Predator–prey relationships and responses of ungulates and their predators to the establishment of protected areas: A case study of tigers, leopards and their prey in Bardia National Park, Nepal. Biological Conservation, 142(1), 189–202. https://doi.org/10.1016/j.biocon.2008.10.020

Whiteley, A. R., Fitzpatrick, S. W., Funk, W. C., & Tallmon, D. A. (2015). Genetic rescue to the rescue. Trends in Ecology & Evolution, 30(1), 42-49. https://doi.org/10.1016/j.tree.2014.10.009

How to cite this article: Hill E, Linacre A, Toop S, Murphy N, Strugnell J. Widespread hybridization in the introduced hog deer population of Victoria, Australia, and its implications for conservation. Ecol Evol. 2019;9:10828–10842. https://doi.org/10.1002/ece3.5603