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Tarantulas (Araneae: Theraphosidae) in the pet trade in South Africa

Tinyiko C Shivambu^{1,2*} , Ndivhuwo Shivambu^{1,2} , Robin Lyle³ , Adriaana Jacobs³, Sabrina Kumschick^{4,5} ,
Stefan H Foord⁶  and Mark P Robertson² 

¹ Centre for Invasion Biology, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, South Africa

² Centre for Invasion Biology, Zoology and Entomology, University of Pretoria, Pretoria, South Africa

³ Biosystematics Division, Agriculture Research Council-Plant Protection Health and Protection, Pretoria, South Africa

⁴ Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Stellenbosch, South Africa

⁵ South African National Biodiversity Institute, Kirstenbosch Research Centre, Cape Town, South Africa

⁶ Department of Zoology and Centre for Invasion Biology, University of Venda, Thohoyandou, South Africa

*Correspondence: shivambucavin@gmail.com

Many alien species have been introduced around the world as part of the pet trade, and some have escaped captivity and become invasive. In South Africa, many species of tarantula (Theraphosidae) are kept as pets. It is not known which species are traded, which are most popular, and whether their names are correctly applied. Online traders and physical pet stores were investigated between 2015 and 2016 to determine the extent or size of trade, species composition, most popular species, and their invasion history elsewhere. In total, 36 specimens, three individuals from 12 putative species, were also purchased for DNA barcoding targeting the COI gene region to quantify the accuracy of tarantula identification by traders. In total, 195 tarantula species were advertised for sale, and the most popular species were *Brachypelma albopilosum* Valerio, 1980 ($n = 199$), *B. vagans* Ausserer, 1875 ($n = 132$), and *Grammostola rosea* Walckenaer, 1837 ($n = 120$). The composition of shared species differed between the sources and most of the species were advertised online. Only one of the popular species, *B. vagans*, has been recorded as being invasive elsewhere. Only 36% of the barcoded specimens matched existing barcodes in online repositories that had the same species name. The three individuals from 12 putative species were not in the same terminal clade as those of conspecifics in the Barcode of Life Data System (BOLD) and the NCBI GenBank reference sequences. A large proportion of the known tarantula species are traded in South Africa and must be included in management and risk assessments to avoid potential invasions.

Keywords: alien species, biological invasions, e-commerce, establishment success, COI, DNA barcoding

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Introduction

Keeping wild animals as pets has become a popular hobby that has increased around the world (Kopecký et al. 2013; Mori et al. 2017). Many of these species are traded for entertainment (da Nóbrega Alves et al. 2010), profit (van Wilgen et al. 2008; Mori et al. 2017) and ornamentation (Murray et al. 2012; Kopecký et al. 2013). The internet has increased the opportunity for trade in live animals (Mendiratta et al. 2017). A range of taxa including spiders (Yen and Ro 2013; Hauke and Herzig 2017), amphibians (Measey et al. 2017), birds (Mori et al. 2017), small mammals (Lankau et al. 2017) and reptiles (van Wilgen et al. 2010) are frequently traded in this way.

The pet trade has become an important pathway for the introduction of non-native species to new regions (van Wilgen et al. 2008; Hulme 2015; Measey et al. 2017; Mori et al. 2017). These pets may accidentally escape from captivity (Mazzotti and Harvey 2012), but the deliberate release of pets by owners is recognised as a major problem (Hulme 2015; Stringham and Lockwood 2018; Maceda-Veiga et al. 2019). Introduced pet species are released into the wild for several reasons, including

aggressiveness, fear of zoonotic diseases, unwanted gifts (Padilla and Williams 2004; Reaser and Meyers 2007) or because they become too large to keep (Holmberg et al. 2015). Many species that escape, or are released, may be unable to survive and establish self-sustaining populations (Zenni and Nuñez 2013). However, those species that successfully establish and become invasive can have severe environmental and economic impacts (Nelufule et al. 2020; Shivambu et al. 2020a, 2020b). Some of the species damage ecosystems (Martin and Coetzee 2011), compete for resources with native species (Mori et al. 2017; Nunes et al. 2017) and carry pathogens that threaten public health (Travis et al. 2011) and agriculture (Witmer and Hall 2011; Gibson and Yong 2017), and cause biodiversity loss (Engeman et al. 2007; Faulkes 2010).

A recent review by Lockwood et al. (2019) highlights the urgent necessity to understand better how the exotic pet trade contributes to invasions in order to devise strategies to limit its harmful impacts. An important predictor of whether a species is likely to become invasive in a new region is its history of invasion elsewhere (Hulme 2012),

and this is often used to identify potentially invasive species. Knowing the invasion history of the species may help to assess the likelihood that a species will establish, become invasive, and have an impact (Dextrase and Mandrak 2006; Broennimann and Guisan 2008; Kumschick and Richardson 2013). Consequently, the trade in species that are known to be invasive elsewhere is often regulated by means of legislation (e.g. Maceda-Veiga et al. 2019).

In South Africa, a range of taxa are kept as pets (van Wilgen et al. 2008; van Wilgen et al. 2010; Nunes et al. 2017; Maligana et al. 2020; Shivambu et al. 2020c). Studies have investigated amphibians, reptiles and fishes in the pet trade (van Wilgen et al. 2008; Woodford et al. 2017; Measey et al. 2017), but only one study has explored risks associated with invertebrates (Nelufule et al. 2020). Several species of tarantula (spiders in the family Theraphosidae) are also traded and kept as pets. It is not known how many tarantula species are present in the South African pet trade, their availability (a measure for popularity) or what influences their availability, and whether species composition varies across markets.

As a first step to understanding possible invasion risks associated with the tarantula trade, an accurate inventory of introduced species is required. It is essential to have the correct identity of the species in the pet trade, because it forms the basis for any risk assessment, management and policy development (Moshobane et al. 2019). Problems associated with misidentification of species in the pet trade have been documented (Murray et al. 2012; Arida 2017; van der Walt et al. 2017). Misidentification can result, because somatic characters may often not differ between species, and this is particularly true for juveniles (Hamilton et al. 2011; Turner et al. 2017). Tarantulas in the South African pet trade are likely no exception to this, because traders probably lack the taxonomic expertise required to identify these species (Mendoza and Francke 2017). In addition, common names or trade names (a name consisting of a confirmed genus and common name, e.g. *Avicularia* sp. 'Guyana Blue') are frequently used in the pet trade instead of scientific names. This has the potential to cause confusion, especially when more than one common name is used for a species. Quantifying the extent to which species are misidentified can provide estimates of the diversity of species traded and the true identity of those species.

The identification of specimens by means of DNA barcoding is a popular technique used by taxonomists and conservation biologists, specifically if morphological identification is difficult (Zhou et al. 2016; Mendoza et al. 2017). DNA barcoding is defined as a taxonomic method that uses a short genetic marker in an organism's DNA to identify its affiliation to a particular species (Hebert et al. 2003a). Many studies have used the sequence divergence molecular marker, mitochondrial cytochrome c oxidase subunit I (COI) for DNA barcoding (Hebert et al. 2003b, 2004a, 2004b; Turner et al. 2017). This gene region has been used in the identification of tarantula species, and it has been demonstrated to be more accurate than other molecular markers (Hendrixson et al. 2013; Montes de Oca

et al. 2016). The reliability of using the COI gene region can be tested using DNA barcode gap analysis (Čandek and Kuntner 2015; Liu et al. 2015). A DNA barcode gap is described as the variance between the greatest intraspecific and the smallest interspecific distances (Hebert et al. 2003b). Barcoding using the COI gene region is considered to be reliable, because of the absence of overlap between intra- and interspecific distances, e.g. spiders (Čandek and Kuntner 2015) and marine lobsters (Govender et al. 2019).

The aims of this study were to: 1) determine the size of the trade in tarantulas in the South African pet trade and whether the species composition differs among trade types; 2) determine which species are most commonly available; 3) determine whether any species available have a history of invasion elsewhere; 4) determine whether there is a relationship between availability and selling price; and, 5) quantify the accuracy of identification of a sample of tarantulas in the pet trade.

Materials and methods

Data collection

Sampling

A list of tarantula names was compiled by combining names obtained from online searches, pet store visits and a questionnaire survey (details below). Because common names and trade names predominate in the pet trade, these were matched with common names listed in the Global Biodiversity Information Facility (GBIF 2016) or World Spider Catalog species database (World Spider Catalog 2018) in order to determine their associated scientific names. Species for which there were no matching common and scientific names in these databases were referred to as unconfirmed species (as in Nelufule et al. 2020). We counted the number of times that each species was encountered either online, in physical pet stores or mentioned in the questionnaire survey to generate an availability index value per species. The term 'availability' (following Chucholl and Wendler 2017) was used as a measure of popularity for a particular species.

Online trade searches

To generate a species list of tarantulas in the South African pet trade, several online searches including online pet stores, tarantula forums, tarantula societies, online advertising sites (Gumtree, Ananzi, and Trovit, Junk mail, twitter, and Facebook) and tarantula club websites were undertaken. Searches were undertaken from February to November in 2015 and 2016. Search terms, such as 'tarantula in the pet trade South Africa', 'tarantula for sale in South Africa' or 'pet tarantula in South Africa' were used. Although tarantula species are suspected to be traded on the dark web, this was beyond the scope of the current study.

Physical pet store visits

Pet stores in the provinces of Gauteng (Pretoria [$n = 11$], Johannesburg [$n = 9$]) and KwaZulu-Natal (Durban [$n = 13$]) were visited to obtain a list of tarantula species for

sale. Pet stores in Gauteng were visited in November 2015 and September 2016, whereas those in KwaZulu-Natal were visited in December 2016. Species names, common names, and prices for each species were recorded for each store.

Questionnaire survey

A questionnaire survey, entitled 'South African tarantula survey', was developed to obtain additional information on the tarantula species in the pet trade and to complement the species lists from online surveys and pet store visits. The survey comprised of nine questions and included an online and a paper-based survey. SurveyGizmo was used (<https://www.surveygizmo.com/free-survey-software/>), and the questionnaire was developed based on three types of participants, namely tarantula keepers, sellers and breeders.

The names and the emails of the participants in the survey were optional so that participants could remain anonymous. The survey took roughly 8 min to complete. Flyers were produced to advertise the survey and distributed at the SOS² annual reptile expo (Emperor's Palace, 64 Jones Rd, Kempton Park, in Gauteng province in South Africa on 7 and 8 May 2016) and at the second spring reptile exotic pet expo (Jabulani Recreation Centre, Sandringham in Johannesburg Gauteng province, South Africa on 10 September 2016). Survey flyers were also distributed to pet stores in Gauteng and KwaZulu-Natal provinces and the pet store owners were asked to distribute the flyers to the customers buying tarantulas. Pet store owners who did not want to complete the survey online were given hard copies to complete.

Additionally, the survey link was sent to members of the South African Pet Traders Association (SAPTA: <http://www.sapetraders.co.za/>). The link was also sent through mailbox to the members of advertising sites such as Ananzi, Gumtree, Facebook, OLX and Trovit. The online survey ran for nine months, from April to December 2016. Ethical clearance to conduct this research was approved by the University of Pretoria Ethics Committee (Ethics code: EC160422-0.23).

Statistical analysis

We counted: (1) number of tarantula species in the trade; (2) species that were most popular through online searches, physical pet store visits, and the questionnaire survey; and, (3) number of species advertised via each of the three sources (online stores, physical pet stores and questionnaire survey). The proportion of species shared between sources was assessed. The relationship between species availability and price was statistically tested with linear regression. Prices for species with the same names were averaged. The availability was calculated based on the number of times that each species was advertised for sale. We used the 'specaccum' function in the vegan package (Oksanen et al. 2016) to determine sampling adequacy from the online stores, physical stores and the questionnaire survey. The sampled data were set at 100 random permutations, following the methods of Oksanen et al. (2016). All statistical analyses were performed with R version 3.0.2 (R Core Team 2014).

Species identification

Sampling

To investigate the accuracy of species identifications, we sourced three specimens from each of 12 putative species from different pet stores. Species were selected for DNA barcoding if they: (1) had a high availability score (>100), (2) were morphologically similar and confusing according to the tarantula experts, and (3) were species with trade names only. The specimens were euthanised in 99.99% cold ethanol. The third leg on the right side of specimens was removed at the trochanter. Each specimen and leg was stored in 99.99% ethanol in separate tubes and these were deposited in the Agricultural Research Council - Plant Health and Protection, National Collection of Arachnida (NCA), housed in Pretoria, South Africa.

DNA extraction and PCR

Genomic DNA was extracted from leg tissue (25 mg) using DNeasy® Blood and Tissues Kit following QIAGEN® Kit user manual. Forward (LCO1490) GGTCACAAATCATAAAGATATTGG and reverse (CI-N-2776) 5'-GGATAATCAGAATATCGTTCGAGG-3' primers were used to amplify cytochrome c oxidase subunit I (COI) gene region (Hamilton et al. 2011). In total, 25 µl of PCR reaction contained 2.5 µl 10× Dream Taq buffer, 2 µl dNTPs, 0.5 µl of forward and reverse primers, 0.125 µl Taq (MyTaq DNA Polymerase), and 2.5 µl of DNA and 16.875 µl ddH₂O. The initial denaturation step occurred at 94 °C for 1 min, followed by denaturation at 94 °C for 45 s, and then annealing at 48 °C for 45 s. The extension step occurred at 72 °C for 30 s, and the final extension occurred at 72 °C for 5 min. The PCR products were sent to Inqaba Biotechnical Industries (Pretoria, South Africa) for purification, precipitation, and sequencing.

Molecular analysis

Sequences were manually edited with MEGA version 7.0.26 (Kumar et al. 2016). DNA sequences were aligned with MUSCLE and ClustaW and trimmed to equal length (1 103 bp) without gaps in MEGA. These sequences were compared using BLAST search (Altschul et al. 1997) against the sequence references of the Barcode of Life Data System (BOLD) and the National Center for Biotechnology Information (NCBI) websites to verify the accuracy of species identification. Species with matches greater than 98% were considered as a high match, between 90% and 98% as a medium match and less than 90% as a low match. The percentage matches were classified because few sequences of tarantula species have been deposited into NCBI GenBank.

The reference sequences of the COI gene were downloaded from BOLD and NCBI GenBank. One or two reference sequences linked to each specimen were included, but where the reference sequence was not available, species of the same genus were used. Fourteen reference sequences were included in the phylogenetic tree to compare with the generated sequences of the tarantula specimens (Supplementary material Table S1). The COI sequence of Tuscan bronze, *Aphonopelma vorhiesi* (KY017963.1) downloaded from NCBI GenBank was

included in the phylogenetic tree analysis as the outgroup. The sequence of each tarantula species obtained was deposited to the NCBI GenBank under accession numbers MK213134 - MK274714 (Supplementary material Table S1).

Three phylogenetic analyses, namely maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) were performed using MEGA7 to determine the relationships amongst the specimens. Bootstrap support for MP and ML was set at 1 000 replications. Phylogenetic trees with bootstrap values greater than 70% were retained, because they provide the relationship between terminal taxa (Nei and Kumar 2000). The Bayesian inference (BI) mtDNA phylogenetic tree with posterior probabilities (PP) for 36 sequences was computed in MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001) to test the support for the similarities between the specimens constructed in the ML and MP trees. Bayesian posterior probabilities close to 1 (0.99) support similarities between the taxa (Hamilton et al. 2011; Mwale et al. 2016). Markov Chain Monte Carlo (MCMC) was run for 10 000 000 generations, in order to ensure that the samples produced adequate PP distributions required for BI (Huelsenbeck and Ronquist 2001). Other settings in MrBayes were set at default heating and swap settings. The output tree files were visualised using FigTree version 1.4. jModelTest version 2.1.9 was used to identify the best fit model with fixed topology under the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) (Posada 2008). We performed a barcode gap analysis to determine the reliability of the COI gene region in identifying tarantula species in the pet trade. The intraspecific and interspecific distances were calculated using the Kimura 2-parameter (K2P) model for all the species in MEGA7. The Jeffries-Matusita (J-M) distance statistic was used to determine if intra- and interspecific distances were separable using R statistical software version 3.0.2 (R Core Team 2014). Where the J-M distance was 1.414 or greater, intra- and interspecific distances were considered to be statistically discrete (Trigg and Flasse 2001). A more conservative threshold of 1.90 for the J-M distance was used (Zuzana et al. 2013).

Results

Size of the trade

In total, 195 unique tarantula names were found in the pet trade (Supplementary material Table S2). Of these species, 159 had confirmed scientific names and 36 had unconfirmed trade names (Supplementary material Table S2). The species accumulation curve indicated that sampling from physical pet stores and questionnaire surveys for these species reached an asymptote, but the curve for the online trade did not reach an asymptote (Figure 1). Online and physical pet stores were the sources from which most of the species names were obtained (Figure 2; Supplementary material Table S2). Most species were unique in the online searches (91), with fewer being unique to pet stores (13), and none were unique in the survey (Figure 2). A large proportion of species (25%) were shared amongst all three sources (Figure 2).

In total, 220 common names were used for 195 species by traders (Supplementary material Table S2). In total, 148 species had only one common name each, 17 species

had two common names each, and for 30 of the species, three or more common names were used for each species (Supplementary material Table S2). From online trade alone, four unconfirmed common names ('Borneo big black', 'Brazilian light pink bloom', 'Ecuadorian red blue' and 'Ghost big tree') were used by traders to identify species without the scientific names.

Most available species

The top five species with highest availability across all three sources combined were *Brachypelma albopilosum* ($n = 199$), *B. vagans* ($n = 132$), *Grammostola rosea* ($n = 120$), *Nhandu tripepii* Dresco, 1984 ($n = 103$), and *Grammostola pulchripes* Simon, 1891 ($n = 101$) (Supplementary material Table S3). The availability of species sampled differed across the three sources (Supplementary material Table S3). When the top 10 and top 30 most available species were compared across the three sources, the proportion of shared species was low (<0.5) to moderate (~0.8, Figure 3). The proportion of the top 10 and top 30 most available species shared between 2015 and 2016 was low, suggesting considerable turnover between years (Figure 3).

Invasiveness elsewhere

Only one species reported in the South African pet trade, *Brachypelma vagans*, has been recorded as being invasive elsewhere (Supplementary material Table S4). It has been reported to be in two stages of invasion, i.e. introduced and established, with the pet trade recorded as one of the modes of introduction (Supplementary material Table S4). In addition, climate matching and distribution modelling for *B. vagans* was performed to determine the areas that may be suitable should the species be released or escape captivity (see methods in Supplementary material). The model performance was tested for this species, and the jackknife test indicated that the models did not perform well in predicting the suitable areas for *Brachypelma vagans* in South Africa (proportion predicted correctly >0.846; $p > 0.05$). However, the predicted climatic suitability for this species only occurred in small areas in the southwestern coastal areas of the Western Cape province and largely in Namibia (Supplementary material Figure S1). The climatic zones of the native range of *B. vagans* matched climate zones in South Africa, where each zone exhibited small areas of similar climate (Supplementary material Figure S2). The climatic match covers the portion of inland areas within the Eastern Cape and western interior extending towards the coastal areas (Supplementary material Figure S2).

Relationship between price and species availability

Price was found to be a poor predictor of availability, because the relationship was found to be significant only in certain cases and the r^2 values were low in all cases (Figure 4; Supplementary material Figure S3). Availability tended to be higher for cheaper species with the most highly available species usually priced below ZAR 500.00 (Figure 4). Prices for species ranged from ZAR 15.00 to ZAR 7 120.00 (mean \pm SD; 295.51 \pm 286.62). *Theraphosa stirmi* Rudloff and Weinmann, 2010 (average = ZAR 7 120.00) was the most expensive species, whereas *Haplopelma longipes* von Wirth

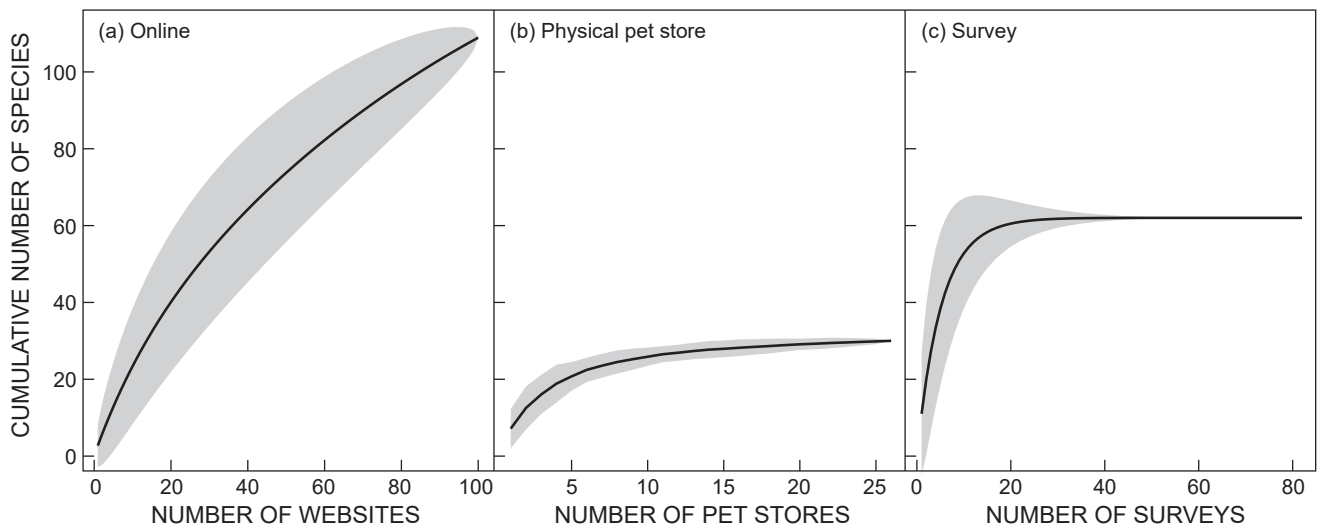


Figure 1: Species accumulation curves demonstrating the number of tarantula species in the South African pet trade data that were obtained from: (a) online searches, (b) physical pet stores visits and (c) through questionnaire surveys. The black line is the mean species accumulation curve and the grey shading is the standard deviation from 100 random permutations of the data

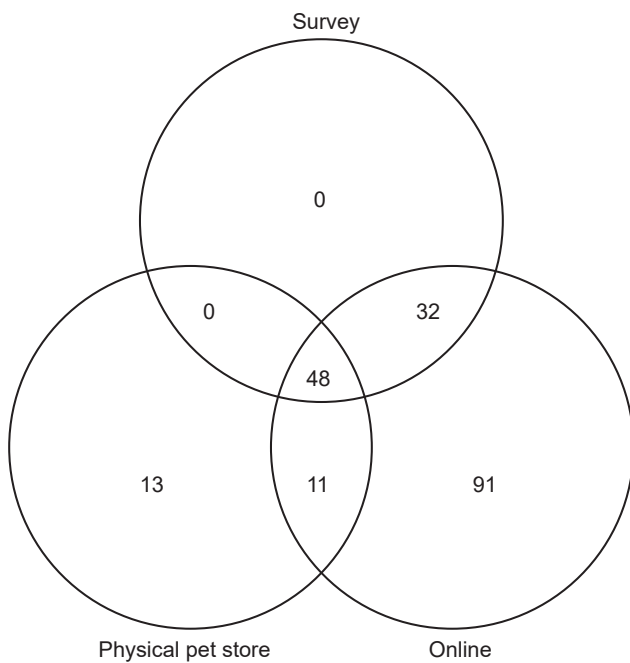


Figure 2: A Venn diagram showing the total number of confirmed tarantula species obtained from three different sources and those shared among the sources. The sources include questionnaire surveys, online searches, and physical pet stores

and Striffler, 2005 (average = ZAR 30.00) and *Grammostola porteri* Mello-Leitão, 1936 (average = ZAR 70.00) were the cheapest species (Supplementary material Table S5).

Species identification

Only seven COI reference sequences representing seven species were available in the NCBI GenBank database for the 12 species selected in this study (Table 1). Of the 36 specimens, 13 matched with barcodes of species

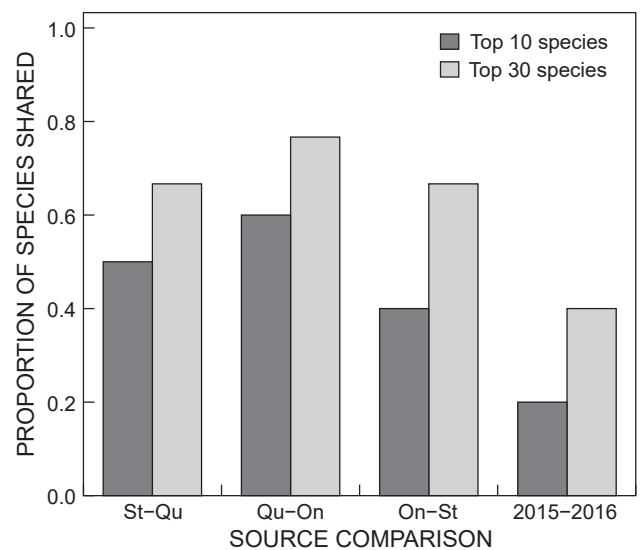


Figure 3: The proportion of species shared between different sources, including the questionnaire survey (Qu), the pet stores (St) and online searches (On) for species ranked in the top 10 and for the top 30 of availability. The proportion of species shared for the top 10 and top 30 ranked species for online searches in 2015 and 2016 is also shown

with high similarity defined at 98%, whereas eight had high similarity with species of the same genus defined at 98%, and three with similarity defined at 91% (Table 1). The remaining 15 specimens matched with species from different genera, 13 had high similarity at 98%, two at 93% and one low similarity at 85% (Table 1).

The Tamura 2-Parameter (T2P) was selected as the best-fit model, and the tree topologies were similar for MP, ML, and BI analyses (Figure 5). The sequences of tarantula specimens resulted in a partial 1 103 bp for the COI region. The most parsimonious tree length was 1 114,

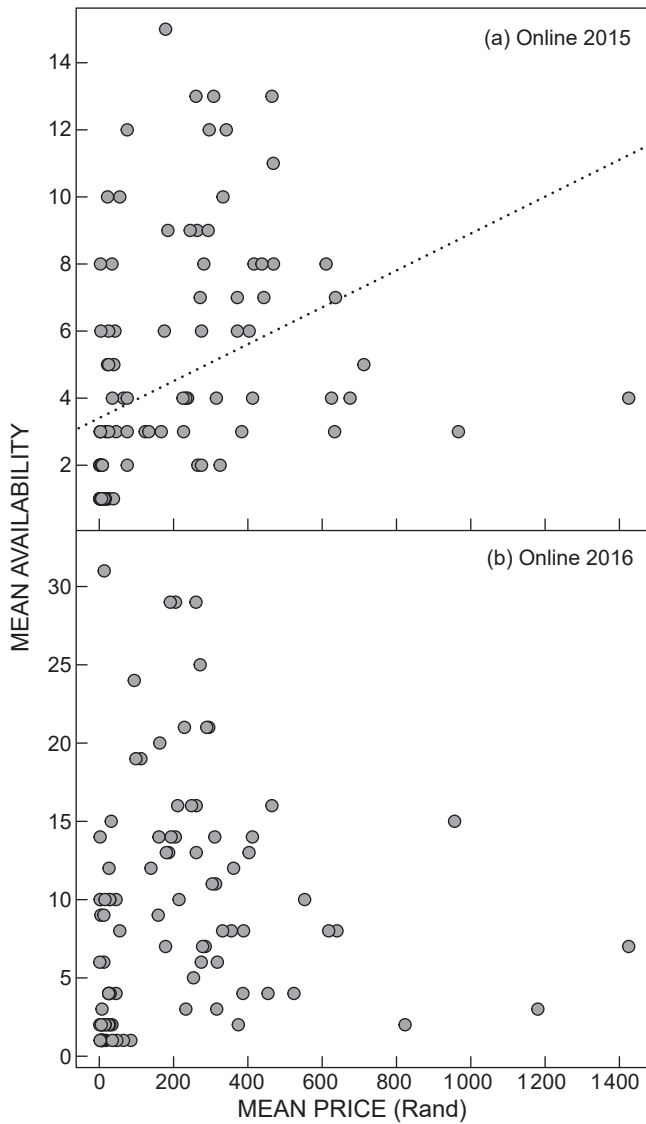


Figure 4: The relationship between mean tarantula availability and mean price (in South African Rand) for species advertised online. (a) Represents online stores in 2015 ($p = 0.0056$) and (b) online stores in 2016 ($p = 0.25$). The black dotted line represents the regression line. The availability is the number of times that each species was advertised for sale in the pet trade

the consistency index was 0.428, retention index (0.811) and the composition index was 0.453 for all sites. The generated sequences obtained from pet shop specimens produced a fully resolved phylogenetic tree with strong nodal support values (ML >70%, MP >73%, BI >0.93) (Figure 5). The phylogenetic analysis formed two major clades, of which only one was well supported. The first major clade comprised of 35 specimens of tarantulas separated into two clades, both with low nodal support values. The second major clade comprised of only one specimen, *A. avicularia* (specimen: 16), which was well supported.

We expected specimens sold under the same name from each of the 12 putative species to have high NCBI BLAST percentage matches with the species of the same

name and to be clustered together with high nodal support values. However, none of the three specimens from each of the 12 putative species was clustered together (Figure 5). However, two pairs were obtained with specimens, such as *Poecilotheria regalis* Pocock, 1899 (15 and 32), *Cyclosternum fasciatum* Pickard-Cambridge, 1892 (18 and 22), *Lasiadora parahybana* Mello-Leitão, 1917 (27 and 28), and *Brachypelma albopilosum* (5 and 30) forming sister groups (Figure 5). Specimens from different genera, such as *Grammostola pulchripes* (10) with *P. regalis* (13) and *B. albopilosum* (33) with *N. tripepii* (7) clustered together with high nodal support values (ML ≥ 97 , MP ≥ 80 , BI ≥ 0.99) (Figure 5). Other specimens formed clades with specimens of the same genus, e.g. *Lasiadora klugi* (29) with *L. difficilis* Mello-Leitão, 1921 (17) and *L. difficilis* (21) with *L. parahybana* (24) with high support values (ML ≥ 99 , MP ≥ 99 , BI ≥ 0.97). Only 14 specimens out of 36 formed clades with the reference sequence of the same names with high nodal support values (ML ≥ 90 , MP ≥ 89 , BI ≥ 0.93) (see the clustered group in bold in Figure 5). Only one specimen was clustered with the reference sequence of a different species, whereas the remaining 21 (58%) were not clustered with the reference sequence.

The frequency graph showed that there was no barcode gap between the intra- and interspecific K2P distances, and an overlap was found between the two distances (Supplementary material Figure S4). The overlap between intra- and interspecific distances ranged from 0.06 to 0.12 (Supplementary material Figure S4). The intraspecific values ranged from 0.011 to 0.184, whereas interspecific values ranged from 0.064 to 0.20 (Supplementary material Figure S4). The intraspecific mean was lower (mean \pm SD; 0.123 ± 0.05 , $n = 12$) than the interspecific mean K2P distance (mean \pm SD; 0.153 ± 0.24 , $n = 66$). The Jeffries–Matusita (J–M) distance statistic was 0.358; this implies that the intra- and interspecific distances for tarantula species are not statistically separable, because the calculated value was lower than 1.414 and 1.90, which have previously been used as thresholds (Trigg and Flasse 2001; Zuzana et al. 2013).

Discussion

At least 195 species of tarantula are sold in South Africa, which represents 20% of the known tarantula species worldwide (Gallon 2000). The species accumulation curves for tarantula species showed no sign of reaching an asymptote for one of the three sources, which indicates that many species were not sampled, and the list of species gathered is likely to be an underestimate of the total number of species. One of the difficulties experienced in this study is that most of the species were traded under common names and several were under trade names rather than scientific names, which meant that several species could not be linked to verified species names. This results in difficulties in obtaining accurate estimates of the size of the trade. A study by van Wilgen et al. (2010) also found a large number ($n = 266$) of alien reptile species in the South African pet trade. However, only a few amphibians seem to be traded in the country including three species of frogs, namely *Hyperolius marmoratus*,

Table 1: Species identification results from BLAST™ search of 36 tarantula specimens' sequences obtained from pet stores. Specimen names (name that each species was allocated during the sale) were deposited in the NCA (ARC) and each species assigned an accession number. Specimen's ID is the identification code used to recognise the species during analysis. COI (Cytochrome c oxidase subunit I) is the gene region with high similarities matches of 36 tarantula specimens in the pet trade to the sequences from NCBI GenBank and BOLD with their accession number. Species with BLAST™ match >98% was considered high, between 90% and 95% medium and <90% as low BLAST match

ARC Accession No.	Specimens ID	Specimen name	Base pairs (bp)	COI (%BLAST™ match)	Accession number
NCA 2017/418	3	<i>Avicularia avicularia</i>	1 103	<i>Lasiadora parahybana</i> (93)	JN018128.1
NCA 2017/419	8	<i>Avicularia avicularia</i>	1 103	<i>Brachypelma albopilosum</i> (97.3)	TARWO053-10
NCA 2017/407	16	<i>Avicularia avicularia</i>	1 103	<i>Avicularia avicularia</i> (99.5)	MG273535.1
NCA 2017/406	6	<i>Acanthoscurria geniculata</i>	1 103	<i>Acanthoscurria geniculata</i> (100)	TARWO090-11
NCA 2017/389	20	<i>Acanthoscurria geniculata</i>	1 103	<i>Nhandu chromatus</i> (98.4)	TARWO065-10
NCA 2017/417	35	<i>Acanthoscurria geniculata</i>	1 103	<i>Lasiadora parahybana</i> (98)	JN018128.1
NCA 2017/392	5	<i>Brachypelma albopilosum</i>	1 103	<i>Brachypelma albopilosum</i> (97.3)	TARWO053-10
NCA 2017/393	30	<i>Brachypelma albopilosum</i>	1 103	<i>Brachypelma albopilosum</i> (97.3)	ARWO053-10.
NCA 2017/394	33	<i>Brachypelma albopilosum</i>	1 103	<i>Grammostola rosea</i> (99.8)	GBMIN115307-17
NCA 2017/408	11	<i>Brachypelma vagans</i>	1 103	<i>Brachypelma vagans</i> (99.2)	GBMIN115338-17
NCA 2017/390	26	<i>Brachypelma vagans</i>	1 103	<i>Brachypelma vagans</i> (99)	AJ584634.1
NCA 2017/391	31	<i>Brachypelma vagans</i>	1 103	<i>Brachypelma</i> sp. SJL-2007 (100)	AJ584637.1
NCA 2017/409	18	<i>Cyclosternum fasciatum</i>	1 103	<i>Davus</i> sp. DO-2015 (97)	KP757183.1
NCA 2017/395	22	<i>Cyclosternum fasciatum</i>	1 103	<i>Davus</i> sp. DO-2015 (91)	KP757183.1
NCA 2017/396	23	<i>Cyclosternum fasciatum</i>	1 103	<i>Grammostola rosea</i> (99.6)	GBMIN115307-17
NCA 2017/410	10	<i>Grammostola pulchripes</i>	1 103	<i>Grammostola rosea</i> (91.3)	GBMIN113972-17
NCA 2017/397	25	<i>Grammostola pulchripes</i>	1 103	<i>Lasiadora parahybana</i> (100)	JN018128.1
NCA 2017/398	34	<i>Grammostola pulchripes</i>	1 103	<i>Eupalaestrus campestratus</i> (99.8)	AGB004-11
NCA 2017/399	2	<i>Grammostola rosea</i>	1 103	<i>Grammostola rosea</i> (99.6)	GBMIN115307-17
NCA 2017/411	9	<i>Grammostola rosea</i>	1 103	<i>Acanthoscurria geniculata</i> (99.8)	TARWO090-11
NCA 2017/400	14	<i>Grammostola rosea</i>	1 103	<i>Grammostola rosea</i> (99.8)	GBMIN115307-17
NCA 2017/420	17	<i>Lasiadora difficilis</i>	1 103	<i>Lasiadora parahybana</i> (99)	JN018128.1
NCA 2017/421	21	<i>Lasiadora difficilis</i>	1 103	<i>Lasiadora parahybana</i> (100)	JN018128.1
NCA 2017/401	36	<i>Lasiadora difficilis</i>	1 103	<i>Brachypelma albopilosum</i> (97.3)	TARWO053-10
NCA 2017/412	12	<i>Lasiadora klugi</i>	1 103	<i>Davus</i> sp. DO-2015 (95)	KP757183.1
NCA 2017/412	19	<i>Lasiadora klugi</i>	1 103	<i>Lasiadora parahybana</i> (89)	JN018128.1
NCA 2017/403	29	<i>Lasiadora klugi</i>	1 103	<i>Lasiadora parahybana</i> (99)	JN018128.1
NCA 2017/413	24	<i>Lasiadora parahybana</i>	1 103	<i>Lasiadora parahybana</i> (100)	JN018128.1
NCA 2017/422	27	<i>Lasiadora parahybana</i>	1 103	<i>Lasiadora parahybana</i> (98)	JN018128.1
NCA 2017/414	28	<i>Lasiadora parahybana</i>	1 103	<i>Lasiadora parahybana</i> (98.1)	JN018128.1
NCA 2017/404	1	<i>Nhandu tripepii</i>	1 103	<i>Lasiadora parahybana</i> (88)	JN018128.1
NCA 2017/423	4	<i>Nhandu tripepii</i>	1 103	<i>Brachypelma</i> sp. SJL-2007 (100)	AJ584637.1
NCA 2017/417	7	<i>Nhandu tripepii</i>	1 103	<i>Grammostola rosea</i> (99.8)	GBMIN115307-17
NCA 2017/424	13	<i>Poecilotheria regalis</i>	1 103	<i>Aphonopelma hentzi</i> (85)	JF803354.1
NCA 2017/416	15	<i>Poecilotheria regalis</i>	1 103	<i>Poecilotheria regalis</i> (99.3)	TARWO047-10
NCA 2017/405	32	<i>Poecilotheria regalis</i>	1 103	<i>Poecilotheria regalis</i> (99.7)	TARWO103-11

Sclerophrys gutturalis, and *Xenopus laevis* (Measey et al. 2017), all of which are native to parts of South Africa. Nelufule et al. (2020) reported a total of 53 species (36 confirmed and 17 unconfirmed) of terrestrial invertebrates (excluding tarantulas) in the South African pet trade. It is clear that tarantulas dominate the trade in terrestrial invertebrates in South Africa, at least by numbers of species. Most of the terrestrial invertebrate species listed by Nelufule et al. (2020) are used as food for pets, whereas a small proportion of scorpions and insects were kept as pets. They suggest that trade in other pet taxa, such as reptiles and possibly tarantulas may be driving the demand for other terrestrial invertebrates that are used as feeders.

Of the three sources of data in this study, the largest number of species was obtained from online sources, followed by physical pet stores and the questionnaire survey. The internet was also found to be one of the sources with the largest number of parthenogenetic marbled

crayfish (*Procambarus* sp.) in North America (Faulkes 2010). There has also been an increase in person-to-person trade of many commodities worldwide, including the trade in pets (Faulkes 2010; Derraik and Phillips 2010). There were differences in the composition of species between online and physical pet stores, and there were species that were unique to physical pet stores that were not recorded in the online trade. Given these differences in species composition among sources, it is necessary to record species from multiple sources in order to obtain a comprehensive understanding of the pet trade in South Africa, but the same is probably true for other regions.

Five species, including *Brachypelma albopilosum*, *B. vagans*, *Grammostola rosea*, *Nhandu tripepii*, and *G. pulchripes* had the highest availability in all sources. The availability of species in the pet trade is linked to the demand for the species (Patoka et al. 2014; Chucholl and Wendler 2017). It has been reported that high availability of

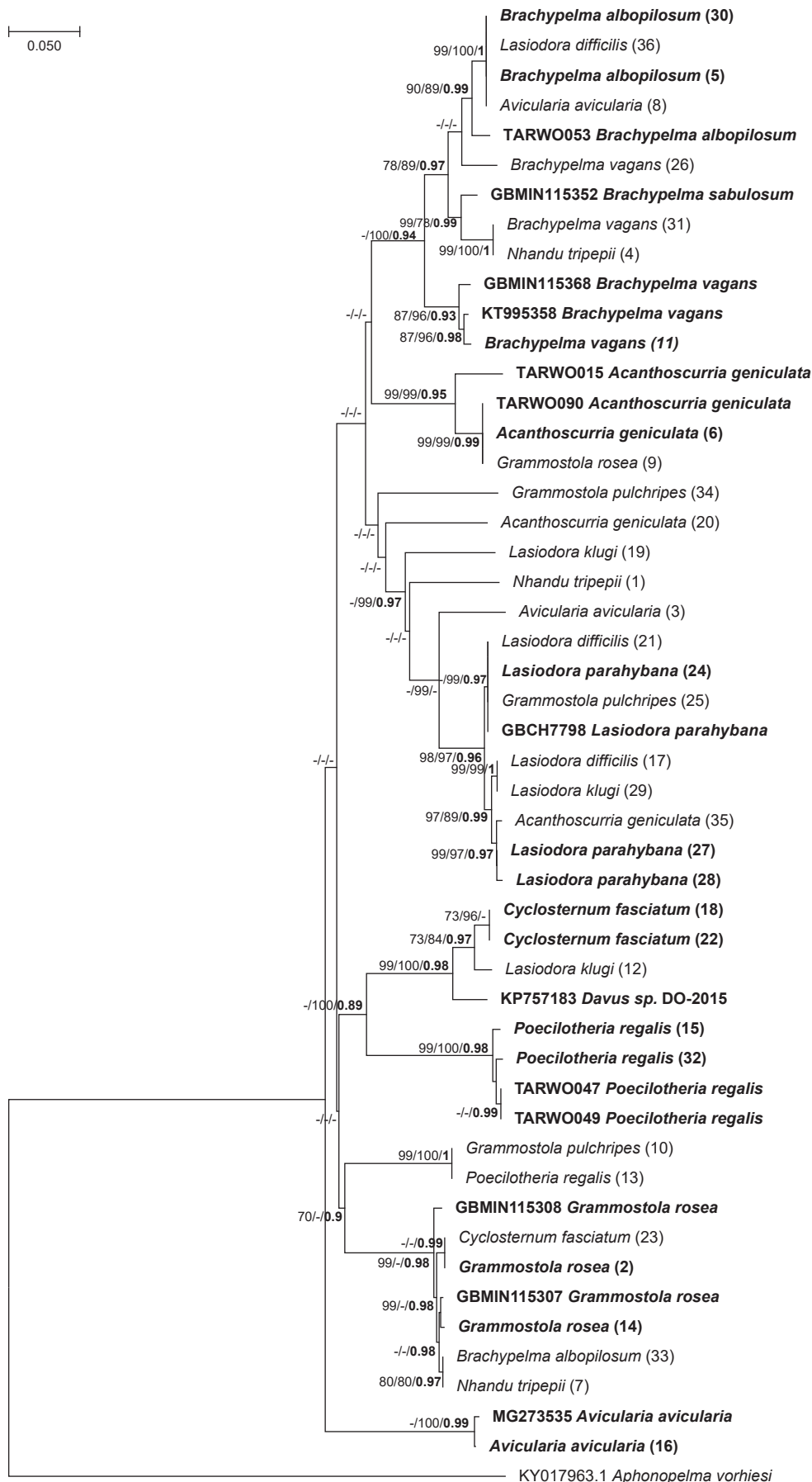


Figure 5: Cladogram of 36 sequenced tarantula specimens purchased in South African pet stores in 2016. The values in the tree branch are the bootstrap percentage values of maximum parsimony, maximum likelihood and Bayesian posterior probabilities of 1. The bootstrap replicate was set at 1 000 for each tree, and the values less than 70 were represented by dashes. The sequence divergence of 1 103 base pairs (bp) of the COI gene region calculated using Tamura 2-Parameter (T2P) model. Species with accession numbers are the reference sequences, and *Aphonopelma vorhiesi* (Tuscan bronze) was used as an outgroup. The numbers in brackets are the specimen identifiers (Table 1) used to discriminate species purchased from the pet shops. Bold indicates species that formed sister groups

species in the pet trade often results in increased 'release pressure', which is defined as the discharge of species from captivity for various reasons, which might include the burden or stress caused by the pet (Cassey et al. 2004; Kikillus et al. 2012). It is known for many introduced pet species that the higher the species availability, the greater the chance that the species will escape or be released, and subsequently may establish widely (Nunes et al. 2017; Stringham and Lockwood 2018; Maceda-Veiga et al. 2019).

It is not known what influences the availability of tarantula species in the South African pet trade. However, the results indicated that price is not a good predictor of species availability and therefore, other variables are likely to explain the variation. Although the price was not a reliable predictor, the species with high availability were generally priced below ZAR 500.00. Similarly, the studies by Su et al. (2015) and Vall-Ilosera and Cassey (2017) showed that most abundant pet birds were sold at lower prices, whereas the least abundant species were priced higher. As a result, abundant species sold at lower prices are released more frequently than rare and expensive species (Stringham and Lockwood 2018; Maceda-Veiga et al. 2019).

One of the top three most available species in all three sources and one of the cheapest species in the South African pet trade, *Brachypelma vagans*, has become established in Belize and Florida in the United States of America (Edwards and Hibbard 1999, 2003; Reichling 2000). This species was reported to have been introduced through the pet trade and released from captivity in Florida (Thomas 1995; Edwards and Hibbard 1999, 2003). Some parts of South Africa seem favourable in terms of climatic suitability and could allow the species to establish, should it be released or escape from captivity. However, the risk of invasion is probably low for *B. vagans* given that areas of suitable climate in South Africa are small. However, it is not only species that have become invasive elsewhere that could be problematic, and it is important to consider other factors as part of a risk assessment (e.g. Roy et al. 2018).

Scientific names are important when applying risk assessments, because they link to the literature about the biology of the species (Schlaepfer et al. 2005; van Riemsdijk et al. 2017). The almost exclusive use of trade names and common names in the tarantula trade can cause challenges for determining the scientific names of these species. Several trade names ($n = 36$) could not be linked to verified species names in GBIF (2016) and the World Spider Catalog (World Spider Catalog 2018). Seventeen species had two common names each and 30 species had three or more common names each, so some common names are shared amongst the species and preferred over scientific names. This is not specific to the tarantula trade. Lankau et al. (2017) found that rodents were sold online using common names only. According to the tarantula traders, common names are easy to remember, because they depict species morphological characters, e.g. colour of the body parts (*Avicularia avicularia* - Pinktoe) and size of the species (*Nhandu tripepii* - Brazilian giant blonde). The use of common names complicates the application of correct scientific names, which could result in species misidentification.

Species in the pet trade cannot be regulated or monitored effectively unless their true identity is known (Schlaepfer et al. 2005; Sanders et al. 2008). In this study, most of the species were incorrectly identified by traders. This indicates that the accuracy of identified tarantula species in the pet trade is poor because only 14 specimens out of 36 clustered with reference species of the same taxa. This may also indicate that names used for tarantula species in the pet trade may be incorrect. This could be solved with traditional taxonomic identification for adults (Petersen et al. 2007) or DNA barcodes, as demonstrated in this study. For example, some of the species from the genera *Acanthoscurria*, *Avicularia*, *Brachypelma* and *Lasiadora* were clustered together in this study. Previous studies indicated that these species are morphologically similar (Figure 6) and therefore difficult to identify (West 2005; Ferretti et al. 2011; Gonzalez-Filho et al. 2012; Bertani and Huff 2013). The study by Turner et al. (2017) explored the relationship between tarantula species of different tribes (e.g. *Grammostolini*, *Hapalopini*, and *Theraphosini*), and these tribes are represented as closely related in the South African pet trade. This could explain the incorrect identifications and scientific nomenclature used in the pet trade.

Various factors can contribute to misidentifications. For example, juveniles and females are often impossible to identify to species level, because there is insufficient knowledge on how to discriminate certain species morphologically, and there may be hybrids (Stuart and Parham 2007; Natusch and Lyons 2012). These issues are not specific to tarantulas. Identification of species in the pet trade is complex, and misidentification is frequent, for example, this has been reported for snakes and seahorse species (Zhou and Jiang 2005; Sanders et al. 2008; Zeng et al. 2019). Different species that have a similar appearance might be kept together, which may result in hybridisation (Natusch and Lyons 2012; van der Walt et al. 2017). Scientific evidence to suggest hybridisation in the pet trade is however lacking. Schultz and Schultz (2009) and Turner et al. (2017) confirmed that tarantulas could hybridise, including closely related *Aphonopelma* and *Brachypelma* species. Turner et al. (2017) also suggest that there has been recent radiation in the *Brachypelma* clade, and this could explain why they hybridise. Furthermore, the taxonomy of several tarantula groups is not well resolved (Hamilton et al. 2011; Mendoza et al. 2017; Turner et al. 2017). Recent taxonomic revisions have been undertaken for the genera *Pamphobeteus* (Bertani et al. 2008), *Aphonopelma* (Warriner 2008), *Grammostola* (Ferretti et al. 2011), *Avicularia* (Bertani and Huff 2013) and *Brachypelma* (Mendoza et al. 2017).

This study reported a small number of specimens that matched that of the same taxa in the BOLD or NCBI databases. DNA barcoding is straightforward to apply for species that already have reference barcodes deposited to the GenBank (Steinke et al. 2009; Gonçalves et al. 2015). In this study, however, only seven species out of 12 have barcodes available in NCBI GenBank, which has complicated the test of identification accuracy of tarantulas in the pet trade. Hubert et al. (2008) and Pereira et al. (2013) reported that DNA barcoding could identify 90% of



Figure 6: Images of tarantula specimens sampled from the South African pet trade a) *Avicularia avicularia* (specimen: 16), b) *Acanthoscurria geniculata* (6), c) *Brachypelma vagans* (11), d) *Brachypelma albopilosum* (33), e) *Cyclosternum fasciatum* (23), f) *Grammostola pulchripes* (10), g) *Grammostola rosea* (9), h) *Lasiadora difficilis* (17), i) *Lasiadora klugi* (19), j) *Lasiadora parahybana* (27), k) *Nhandu tripepii* (7), and l) *Poecilotheria regalis* (15). The names represented here for each species are original names obtained from the pet trade (numbers in brackets that appear after the name are specimen numbers) (Photograph credits: TC Shivambu)

species that are well analysed, but can fail for incorrectly identified species. One of the limitations of the current study is whether the identity of barcode specimens in the two databases are correct (DeSalle et al. 2005). Our barcode gap analysis showed that there was an overlap between intra- and interspecific genetic distances for tarantula COI sequences. This analysis suggests some limitations to the reliability of the COI gene region for identifying tarantula species (Hebert et al. 2003b, Hebert et al. 2004a; Shen et al. 2016) and this topic warrants additional study. A study by Liu et al. (2015) demonstrated that DNA barcoding was not useful in identifying Chinese viper species. This suggests that the use of other gene regions or additional gene regions could be used to improve the barcoding analysis of tarantula species.

Recommendations

Additional studies should investigate the sale of tarantula species across all South African pet shops. Sampling over a longer period of time (particularly the online

trade) will be crucial to better understand the trade and determine whether any new species have been imported. The factors that could explain the release of tarantula species from captivity should also be investigated, using similar approaches to recent studies (e.g. Maceda-Veiga et al. 2019). Given that most tarantula species were incorrectly identified in this study, priority should be given to establish the identity of unconfirmed species using traditional morphological and DNA barcoding techniques, as suggested by Murray et al. (2012). Considerable effort should also go into sequencing South African theraphosids, so that illegally collected and traded specimens can be accurately identified. Detailed risk analyses (Kumschick et al. 2020) should be conducted on tarantula species in South Africa, particularly those species with high availability and history of invasion elsewhere (e.g. *Brachypelma vagans*).

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ORCID*s*

Tinyiko C Shivambu: <https://orcid.org/0000-0003-3157-4712>
 Ndivhuwo Shivambu: <https://orcid.org/0000-0002-9336-9813>
 Robin Lyle: <https://orcid.org/0000-0002-5279-5306>
 Sabrina Kumschick: <https://orcid.org/0000-0001-8034-5831>
 Stefan H Foord: <https://orcid.org/0000-0002-9195-2562>
 Mark P Robertson: <https://orcid.org/0000-0003-3225-6302>

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