



A new forest dwelling button spider from South Africa (Araneae, Theridiidae, *Latrodectus*)

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Abstract

The medically important spider genus *Latrodectus* Walckenaer 1805, commonly referred to as “button spiders” in South Africa, is represented by six species in the country. Using morphology and the COI barcoding gene we describe a new forest dwelling species, *Latrodectus umbukwane* n. sp. Wright, Wright, Lyle and Engelbrecht. Females have red markings on both the ventral and posterior dorsal surfaces of the abdomen, parallel spermathecae and three loops of the copulatory ducts. Males have an embolus with four loops and diagnostic white markings on the ventral surface of the abdomen that darken with age. Egg sacs are smooth, large, and bright purple when freshly laid, turning shiny grey with time. *Latrodectus umbukwane* n. sp. is known only from sand forest vegetation types in northern Zululand, KwaZulu-Natal, South Africa. A predicted geographic distribution for this species is provided based on cartographic mapping of known habitat and altitudinal preference, from which area of occupancy (AOO; 698 km²) and extent of occurrence (EOO; 4963 km²) were calculated to assess potential IUCN Red List status. Due to the uncertainty of the distribution of this species, a Red List status of Data Deficient (DD) is recommended. An updated key to the southern African species of *Latrodectus* is provided.

Key words: Araneae, Theridiidae, *Latrodectus*, Button Spider, Widow spider, KwaZulu-Natal, Munyawana Conservancy, Phinda Private Game Reserve, sand forest, Tembe Elephant Park

Introduction

Spiders of the genus *Latrodectus* Walckenaer 1805 (Araneae, Theridiidae) are commonly referred to as button spiders in South Africa, redback spiders in Australia, katipo in New Zealand, or widow spiders elsewhere. They are characterised by an absence of teeth on the chelicerae, lateral eyes that are widely separated from the median eyes, and a large colulus (Lotz 1994). All species are considered to be medically important (Maretic 1983; Muller 1993; Garb *et al.* 2004). There are currently 31 recognised *Latrodectus* species globally, with eight recorded from the African continent (World Spider Catalog 2018). *Latrodectus cinctus* Blackwall 1865, *L. indistinctus* O. Pickard-Cambridge, 1904, *L. karrooensis* Smithers 1944, *L. renivulvatus* Dahl 1902, and *L. rhodesiensis* Mackay 1972 are endemic to Africa, and all occur in Southern Africa (Lotz 1994). The brown button spider (*L. geometricus* C. L. Koch 1841) is believed to have originated from Africa or South America, and has been introduced to North America, parts of Europe, parts of Asia, and parts of Australasia (Levi 1959; Garb *et al.* 2004). It is common in parts of southern Africa and is considered cosmopolitan. *L. pallidus* O. Pickard-Cambridge 1872 occurs from the Cape Verde Islands to Libya and *L. tredecimguttatus* (Rossi 1790) from the Mediterranean to China (World Spider Catalog 2018). Both *L. pallidus* and *L. tredecimguttatus* are absent from sub-Saharan Africa (Lotz 1994).

Latrodectus species are difficult to distinguish morphologically. Initially, colour patterns and characteristics of the abdominal setae were used to distinguish between species (Lotz 1994) until Levi (1959) concluded that this was insufficient and recommended using genital morphology instead. Lotz (1994) suggested that the two methods should be used in combination to address morphological overlap in certain species.

Lotz (1994) broadly separated the African species into (1) the *geometricus* species-group (*L. rhodesiensis* and *L. geometricus*) and (2) the *tredecimguttatus* species-group (*L. tredecimguttatus*, *Latrodectus cinctus*, *L. indistinctus*, *L. karrooensis*, *L. renivulvatus* and *L. pallidus*) based on colour and morphological differences including the structure of the female genitalia and male embolus. Garb *et al.* (2004) investigated these relationships using genetic analysis, and suggested two clades: (1) the *geometricus* clade (*L. rhodesiensis* and *L. geometricus*), and (2) the *mactans* clade (all other *Latrodectus* species included in the analysis, including taxa from Africa, the Middle East, Iberian Peninsula, Australia, New Zealand, and the Americas). These results were supported in a separate analysis by Knutson & Miller (2007).

We describe a new species of forest dwelling *Latrodectus* collected in the province of KwaZulu-Natal, South Africa and provide ecological, behavioural, developmental notes, and a phylogeny based on mitochondrial cytochrome oxidase I (COI) to support proposed relationships. An updated key to the southern African species is provided, and data that may be used to diagnose this species from those of other parts of the world are tabulated.

Methods

Females and egg cases were collected from the wild and spiderlings raised in captivity to obtain adult males and developmental data. Spiderlings from four egg sacs were frozen immediately after emergence to determine fecundity. Preserved specimens are deposited in the National Collection of Arachnida at the Agricultural Research Council Plant Health and Protection in Pretoria (NCA), the KwaZulu-Natal Museum, Pietermaritzburg (NMSA), and the Iziko South African Museum in Cape Town (SAMC). Images of specimens in the wild were captured with a Canon Powershot SX 60HS Bridge digital camera. Live habitus images of the adult female were prepared using a Canon 6D with either a Canon L-series 100mm macro or MPE 65mm lens mounted on a Stackshot rail system and stacked using Helicon Focus V6.8.0 software. Spiderlings and immatures were photographed with a Nikon D700 with AF Micro Nikkor 60mm 1:2.8 D lens. Microscopic images were prepared using a high resolution AxioCam MRC5 microscopy camera mounted on a Zeiss Axio Zoom V16 stereo microscope and stacked using the associated ZEN V2.3 SP1 software module. Scanning electron microscope images were prepared with a Phenom Desktop SEM.

Keys for the southern African species are updated from Lotz (1994). Diagnostic morphological data for species from other parts of the world were extracted from the primary literature and tabulated. We use the clade names of Lotz (1994) as they are largely congruent with previous molecular studies and refer specifically to the African fauna.

Potential suitable habitat for the new species was mapped using a combination of unsupervised and supervised classification of Landsat8 imagery captured on the 15th June 2018 to identify potentially suitable habitat in northern KwaZulu-Natal and southern Mozambique. All known localities of the new species occur in sand forest above 60 m in altitude and mapping focused on classification on this habitat type, constrained to this lower altitudinal limit. We consider this a reasonable and conservative approach as 90% of all known stands of sand forest are also found above this altitude (Matthews 2005; Nel *et al.* 2017). Species distribution modelling was not performed due to the very limited number of localities available for the species (van Proosdij *et al.* 2016). Mapping was performed with SAGA (Conrad *et al.* 2015) and QGIS 3.2 software (QGIS Development Team 2019). Definitions for the Area of Occupancy (AOO) and the Extent of Occurrence (EOO) follow IUCN (2012). Climatic data were extracted for the predicted geographic range of the species from the Worldclim 2.0 database (Fick & Hijmans 2017) using the bio5 (mean maximum temperature), bio6 (mean minimum temperature) and bio12 (annual rainfall) bioclimatic variables and summarized.

To further assess validity of the new species we sequenced the barcode region of mitochondrial cytochrome oxidase I (COI) and compared this with publicly available sequences on the Barcode of Life Database (BOLD, <http://www.barcodinglife.org>) and GenBank (<https://blast.ncbi.nlm.nih.gov>). We also conducted a preliminary assessment of the phylogenetic placement of the new species within the genus *Latrodectus* using the same gene region. Total genomic DNA was extracted from two specimens following manufacturer's specifications using a Machery-Nagel

Nucleospin Tissue Kit (Duren, Germany). COI was amplified using the forward primer LC0-1490 and the reverse primer HCO-2198 (Folmer *et al.* 1994). The amplification reaction contained 20 pmol of each primer, a single unit of TakaraTaq (Emerald Amp®MAX HS PCR Master mix, TAKARA BIO INC., Otsu, Shiga, Japan), and 50–100 ng of DNA template made up to 25 µl with distilled water. Cycling conditions were as follows: 94°C for 9 minutes; 35 cycles of 94°C for 45 seconds; annealing at 50°C for 45 seconds and extension at 72°C for 60 seconds with a final elongation step at 72°C for 10 minutes. Positive PCR products were purified using Machery-Nagel NucleoSpin Gel and PCR clean-up kit following the manufactures specifications and thereafter sequenced in both directions using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). Forward and reverse sequences were assembled in CLC Workbench Version 7.9.1 (QIAGEN Aarhus A/S: www.qiagenbioinformatics.com) and trimmed using MEGA7 (Kumar *et al.* 2016). The sequences were deposited in both BoLD and GenBank (MN094888, MN094889). To ascertain the validity of this species the sequences were BLASTed in GenBank and searched in BoLD's species identification tool using the species level barcode records dataset (Ratnasingham & Herbert 2007). Sequences that have a 98% identity match are considered a good indicator of generic or species matches (Altschul *et al.* 1990). MEGA was used to calculate inter-specific pairwise distances.

For phylogenetic analysis all available COI sequences for *Latrodectus* were downloaded from GenBank and BoLD and aligned in MEGA. Sequences that were either identical and/or did not align with our data were excluded, resulting in 91 sequences included in the analysis. Species from the genera *Nesticus* Thorell, 1869 (Nesticidae) and *Synotaxus* Simon, 1895 (Synotaxidae) were chosen as outgroups (but see Fernández *et al.* 2018 for the most recent hypothesis regarding the close relatives of Theridiidae). A Maximum Likelihood analysis was run using Randomized Accelerated Maximum Likelihood v.8.2 (RAxML v.8.2; Stamatakis 2014) under the default general time-reversible model (GTR + G; Tavaré & Miura, 1986). The analysis was repeated twice using the hill-climbing algorithm (Stamatakis *et al.* 2007) and different seeds to ensure that tree space was effectively sampled. For Bayesian analysis the best model of sequence evolution was identified as TPM2uf + I (0.548) + G (0.915) using jModelTest (Posada 2008) based on the Aikake Information Criterion. MrBayes v.3.2.6 (Ronquist & Huelsenbeck 2003) was run twice for 10 million generations, with the rate prior specified as 'variable' and with flat Dirichlet-priors. Tree sampling occurred every 1000th generation, resulting in 10 000 trees of which the first 2500 (25%) were discarded as burn in.

Abbreviations are as follows. *Institutions*: **NCA**: Agricultural Research Council, Plant Health and Protection, Biosystematics Division, National Collection of Arachnida, Pretoria, South Africa; **NMSA**: Kwazulu-Natal Museum, Pietermaritzburg, South Africa; **SAMC**: Iziko South African Museum, Cape Town, South Africa. *Morphology*: **AER**: anterior eye row; **AME**: anterior median eyes; **ALE**: anterior lateral eyes; **PER**: posterior eye row; **PME**: posterior median eyes; **PLE**: posterior lateral eyes.

Taxonomy

Family Theridiidae Sundevall, 1833

Latrodectus Walckenaer, 1805

Latrodectus umbukwane new species Wright, Wright, Lyle and Engelbrecht

Zoobank ID: urn:lsid:zoobank.org:act:AAEDE7DF-8B9D-4853-8BC6-D58E61CD13C0

Type material: Holotype: ♀ (NCA2018/991), **SOUTH AFRICA: KwaZulu-Natal: Umkhanyakude Municipality:** Phinda Private Game Reserve, (27°46'26.40"S 32°20'9.46"E; 64 m.a.s.l.), sand forest, 2017-11-01, B. Wright, from tree hollow, by hand; dissected. **Paratypes:** 2 ♂ (NCA2018/990), same data as holotype, captive raised; 1 ♀ (NMSA29324), same data as holotype, dissected; 3 ♂ (NMSA29325), same data as holotype, captive raised; 1 ♀ (SAMC10028), same data as holotype except Lulubush, Phinda Private Game Reserve (27°47'35.9"S 32°20'33.0"E; 68 m.a.s.l.), not dissected; 3 ♂ (SAMC10029), same data as holotype, captive raised.

Etymology: The species epithet is derived from the isiZulu word "*umbukwane*", meaning something eye-catching or spectacular that one cannot simply walk past it due to its profound beauty. This is in reference to the impressive size and remarkable coloration of this species. It is a noun in apposition and therefore gender neutral.

Measurements: See Tables 1 and 2.

Diagnosis: Females of *L. umbukwane* n. sp. can be separated from African congeners by the combination of a distinct red marking on the ventral surface of the abdomen and a red stripe on the posterior dorsal surface of the abdomen (Figs. 1A–C, 3A, B). Females can further be separated from other African species, except for *L. geometricus* and *L. rhodesiensis*, by having the spermathecae parallel to one another (Fig. 5, 6A, B). They can be distinguished from *L. geometricus* and *L. rhodesiensis* by the following combination of characters: presence of thick curved abdominal setae of approximately the same length as opposed to finer setae of two different lengths in *L. geometricus* and *L. rhodesiensis* (Fig. 4A; Levy 1998); ventral red marking irregular in shape and situated more posteriorly than the orange-red hourglass of *L. geometricus* and *L. rhodesiensis* (Fig. 3B); legs uniformly black in adults and not banded (Fig. 3A, B); abdomen black with light-coloured, irregular oval lines laterally extending onto the dorsal surface with a single dorsal posterior red stripe, positioned medially (Fig. 3A, B); similar pale markings, but more circular in shape, are present along the dorso-median surface of the abdomen; larger size (Table 1). Females can be further distinguished from *L. rhodesiensis* by having three loops of the copulatory ducts as opposed to five (Fig. 5, 6A, B).



FIGURE 1. *Latrodectus umbukwane* n. sp. A–C, Mature female habitus with tenebrionid prey (B) and egg case (C), in situ. D, Sand forest habitat. E, Web retreat on tree branch extending from tree hole. A, D, E, Phinda Private Game Reserve; B, C, Tembe Elephant Park.

Males of *L. umbukwane* n. sp. are generally similar to or slightly smaller than other African species in size (Table 2). They can be diagnosed from all African congeners by having a yellow-white ventral marking anterior to the spinnerets and a yellow-white transverse ventral marking near the book lungs (Fig. 3D). Males can be distinguished from *L. rhodesiensis* by the four loops of the embolus as opposed to five (Fig. 6C–F).

Females of *L. umbukwane* n. sp. can be distinguished from all other species worldwide, except for *L. apicalis* Butler 1877, *L. erythromelas* Schmidt & Klaas 1991, and *L. obscurior* Dahl 1902, by having the spermathecae parallel to one another as opposed to a being in a V-shape (Fig. 5, 6A, B; McCrone & Levi 1964; Kaston 1970; Abalos 1980; Levy 1998; Melic 2000; Knoflach & van Harten 2002; Berendonck & Greven 2005; Vink *et al.* 2008; Aguilera *et al.* 2009; Kananbala *et al.* 2010; Saaristo 2010; Goyal & Malik 2017). *Latrodectus apicalis* and *L. erythromelas* have little published data and no information was found on the shape of their spermathecae. However, *L. erythromelas* can be distinguished from *L. umbukwane* n. sp. by the small size of the adult female (total length 5.5mm-9.0mm). *L. apicalis* can be separated by its geographical distribution as it is considered endemic to the

Galapagos. *L. obscurior* from Madagascar has parallel spermathecae as in *L. umbukwane* **n. sp.** but it is described as being uniformly black in colour with a red ventral marking as in *L. geometricus*, and was previously considered a synonym of this species (Dahl 1902; Levi 1959; Schmidt *et al.* 1994). It is also described as being peridomestic, as is *L. geometricus*, whereas *L. umbukwane* **n. sp.** appears to be limited to pristine sand forest habitats. Therefore *L. umbukwane* **n. sp.** can be distinguished from *L. obscurior* on differences in coloration and habitat selection.

Behavioral traits can be used as additional diagnostic features. *L. umbukwane* **n. sp.** produces a large egg sac, approximately 27 mm long and 17 mm wide, that is bright purple when first laid (Fig. 1C), changing to shiny grey over time. This differs from the smaller, spiked egg sacs of *L. geometricus*, the large, whitish, woolly egg sacs of *L. rhodesiensis*, and the smooth white egg sacs of other African species (Lotz 1994). Fertile egg sacs are generally tear-dropped shaped (n=13), while infertile egg sacs remain rounded (n=3). The webs and selection of retreat sites also appear to be unique amongst African species.

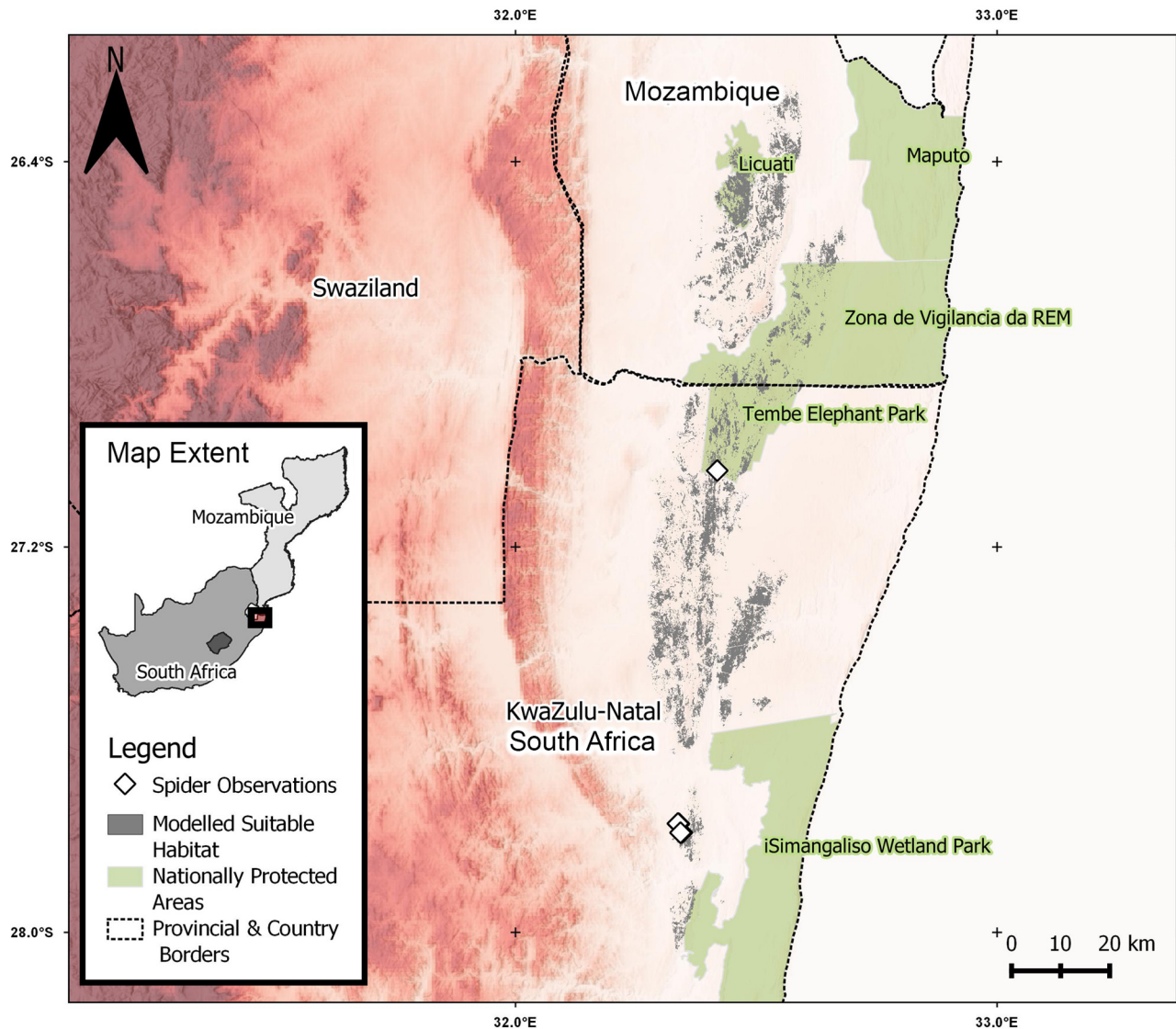


FIGURE 2. Distribution of *Latrodectus umbukwane* **n. sp.** indicating known locality records and extent of potential sand forest habitat in northern KwaZulu-Natal Province, South Africa and southern Mozambique.

Description: *Females:* Measurements in Table 1. Carapace moderately setose on cephalic and lateral parts of thoracic region, less so on posterior part of thoracic region; fovea transverse, procured. Eyes widely separated, all similar in size; PME directly posterior to AME; PLE wider than ALE. Sternum subtriangular. Distal portion of maxillae pallid. Pedipalps with single toothed claw. Legs uniformly moderately setose throughout, tapering distally; metatarsi I and IV gently concave in lateral profile, other segments straight; three tarsal claws present, superior claws with one row of teeth; ventrally situated paired accessory claws present on all legs; tarsus IV with distinct

prolateral row of spines. Abdomen moderately setose with setae all of uniform length. Epigynum variable but oval, elevated and sclerotized with continuous longitudinal procurved opening. Spermathecae dumbbell-shaped, linked by short thick duct; spermatheca 2 larger than spermatheca 1; copulatory openings situated medially; copulatory ducts thinned-walled, weakly sclerotized, coiled around duct between spermathecae and forming three-and-half loops retrolateral to spermathecal (Fig. 5, 6A, B). Coloration of adult in life uniformly black with distinctive red marking ventrally on abdomen situated between spiracles and spinnerets; vertical red stripe present on posterior dorsal surface extending from spinnerets, fading proximally; elongated, irregular white loops on lateral to dorsolateral surface of abdomen; similar but more circular markings are median dorsal surface. *Males*: Measurements in Table 2. Carapace sparsely setose with long setae; fovea transverse, procurved. Eyes proportionally larger relative to carapace than in female, eye pattern as in female except PER closer to AER. Maxillae uniform in colour. Mating bulbs with embolus having four to five coils (Fig. 6C–F), located distally on cymbium, last coil extending medially, curving downwards along retrolateral edge of palp (Fig. 6D), continuing ventrally, curving upwards and terminating in a long thin point; embolus originating retrolaterally, near rounded cymbial apophysis (Fig. 6E); median tegular apophysis sharply bent, almost 90° degrees, ending in blunt point (Fig. 6E). Legs moderately setose with short setae; tarsi lacking accessory claws. Abdomen sparsely covered in elongated setae. Coloration medium to dark brown, cephalothorax paler ventrally. Legs pale brown, darker towards joints and proximal half of femur I dark. Abdomen with white marking ventrally anterior to spinnerets, white longitudinal marking on posterior dorsal surface starting at spinnerets and fading proximally; lateral surfaces with elongated irregular white markings. All white markings with margins darker than the rest of the abdomen. In older specimens these white markings may be absent.

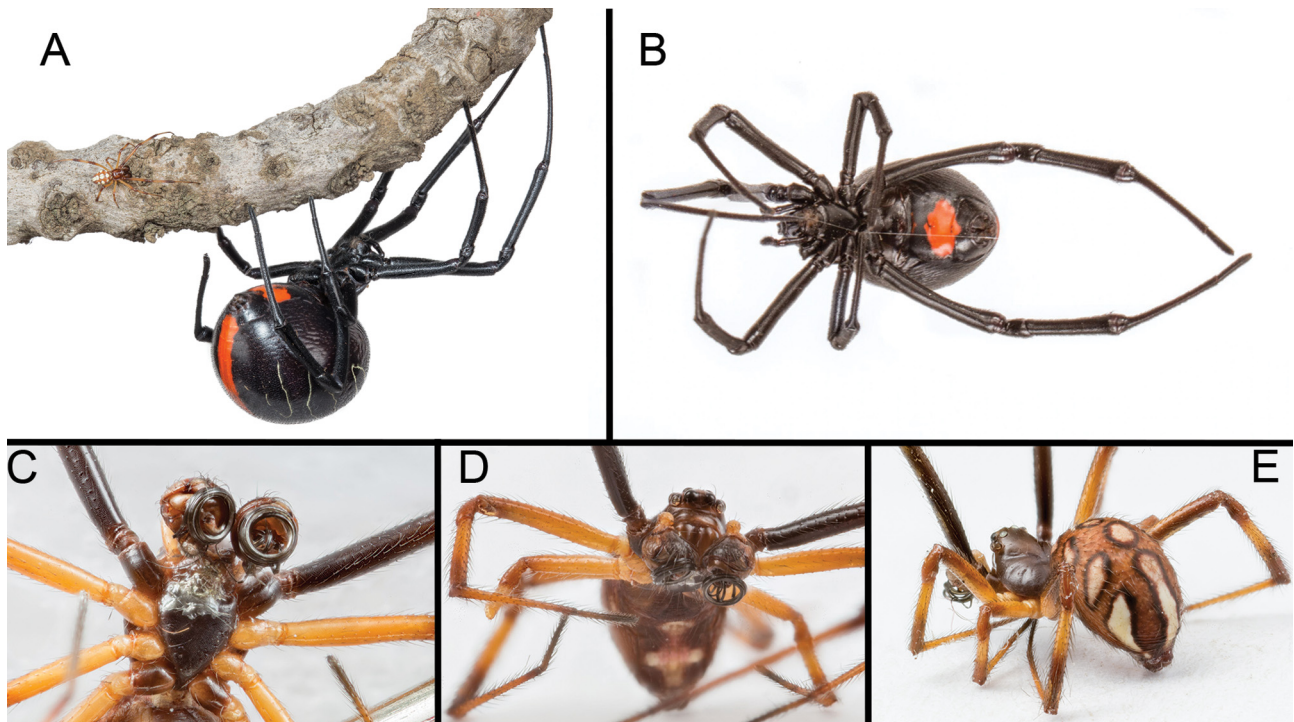


FIGURE 3. *Latrodectus umbukwane* n. sp. live habitus ex situ. A: male (left in image) dorsal view and female lateral view showing posterior dorsal and ventral red markings on abdomen; B: female, ventral view; C: male, ventral view showing pedipalps; D: male, anterior ventral view showing pedipalps and palid ventral marking on abdomen; E: posterior lateral view showing palid dorsal markings on abdomen.

Habitat and conservation status: All known localities for the new species fall within sand forest habitats (Fig. 2). Sand forest is a distinct lowland forest type that is dry, and occurs only on flat terrain (Fig. 1D). It is limited to KwaZulu-Natal province and neighboring parts of Mozambique, and is considered a critically endangered vegetation type (Mucina & Geldenhuys 2006). Of the seven wild female specimens recorded, six were found in pristine, tall sand forest with predominantly bare ground below the canopy, on Phinda Private Game Reserve, which is part of the larger Munyawana Conservancy. A single specimen was found in the garden of the manager's house on Tembe Elephant Park, at the edge of disturbed sand forest. The potential distribution of *L. umbukwane* n. sp. is shown in Figure 2 under the assumption that it is limited to sand forest habitats. The AOO is ca. 698 km² and the EOO is 4963

km². Twenty-three percent of the AOO falls within nationally protected areas in South Africa and Mozambique, and a substantial additional proportion of sand forest is protected in privately protected areas. Assuming the potential distribution developed (Fig. 2) is sufficiently accurate, the extinction risk status for *L. umbukwane* n. sp. would be considered as Least Concern (IUCN 2012). However, given the uncertainty surrounding the ecology of this species, we recommend that a status of Data Deficient is assigned. Additional surveys of nearby coastal forests are required to confirm the assumption that this species is limited to sand forest, but coastal forests have been surveyed for spiders in the past by both professional and citizen arachnologists, and we are not aware of the species having been located during such surveys. Surveys of sand forest should also be a priority to confirm the true extent of occurrence of this species. Climatic conditions across the predicted geographic range of the species are a mean maximum temperature of 32–33.1°C, mean minimum temperature of 10.7–11.6°C, and an annual rainfall of 660–818 mm.

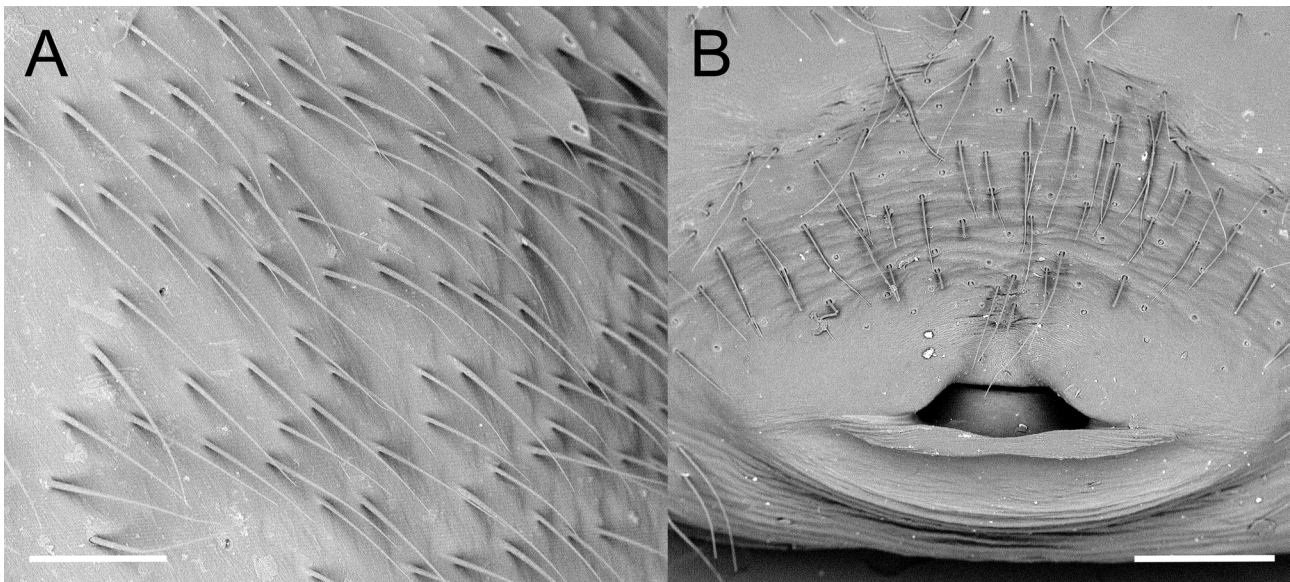


FIGURE 4. *Latrodectus umbukwane* n. sp. female. Scanning electron microscope images of dorsal abdominal setae (A) and epigynum (B). Scale bars 300µm.

The selection of retreat sites also appears to be unique in this species. All specimens located to date were at an elevated position of more than 50 cm above the ground, with a silk retreat in a tree hollow. Webs were not generally present outside the hollow, but when they were recorded, they were typical hackle webs radiating outward from the hollow, and can be extensive (Fig. 1E). Unlike webs of other *Latrodectus* which are closer to the ground with many strands of silk attached to the ground surface, the webs of *L. umbukwane* n. sp. are largely anchored to higher branches, with only a few strands of silk going to ground in a few cases. No sticky silk threads were observed being connected to the ground in the wild or captivity.

Development: Spiderlings (Fig. 7A) typically emerge from the egg sac 29.9 days after laying (27–36 days; n=9). We assume that emerging spiderlings are in the second instar as observed in other *Latrodectus* species (Foster & Kingford 1983). Spiderlings were observed actively moving in some egg sacs for a day or two before emerging, possibly waiting for optimum conditions. Egg sacs produce an average of 584 spiderlings (471–692; n=4). General abdominal shapes, patterns, and positions remain relatively constant in both sexes throughout life, changing only in colour and clarity. Spiderling coloration is golden brown with white dorsal median and lateral abdominal markings on emergence (Fig. 7A). These markings are relatively constant in shape and position for the duration of life, but change in colour and prominence. In third instars the dorsal median and lateral markings remain white but are more prominent (Fig. 7B). In the fourth instar the dorsal median and lateral markings are greenish-white with indistinct dark brown outlines (Fig. 7C). In later instars the dorsal median and lateral markings turn yellow with white outlines, resembling small fried eggs (Fig. 7D). Overall coloration, including the legs which are banded in immatures, darkens until black at maturity after ca. 6 months.

In immature males, the palpal tarsus becomes swollen ca. 15–20 days after emergence. They reach maturity at ca. 26 days. Mature male abdominal coloration fades dramatically as the spider ages. The colour of the abdomen of a young mature male is brown with light yellow-white oblong blotches dorso-medially and laterally, with indistinct dark brown outlines, and no transverse light areas (Fig. 3A). Old mature males have a dark brown abdomen with darker brown irregular circle shapes only visible under bright light or with flash photography.

Phylogenetic relationships: COI sequencing yielded a total of 422 nucleotide base pairs. The identification function on BoLD did not match the COI barcodes to any specimen or species in the database, despite BoLD including sequences for 14 species of *Latrodectus*. The BLAST search on GenBank provided a weak identity match of 87% with a number of genera including *Steatoda* Sundevall 1833, *Crustulina* Menge 1868 and several species of *Latrodectus*. The ML tree is presented with bootstrap support (BS) and posterior probabilities (PP) overlaid (Fig. 7). Two lineages, labelled I and II correspond to the *tredecimguttatus* species-group and the *geometricus* species-group respectively, are supported. *L. umbukwane* n. sp. falls into lineage II. *L. umbukwane* n. sp. appears as a well-supported lineage (BS: 100 %; PP: 1.00), sister to *L. geometricus* (BS: 54 PP: 0.57). The relationship between *L. umbukwane* n. sp. and *L. geometricus* is corroborated by morphological evidence as the two species share parallel spermathecae. The mean inter-specific pairwise genetic distances between *L. umbukwane* n. sp.—*L. geometricus* is 14.7%. Several of the species in lineage I are para- or polyphyletic.

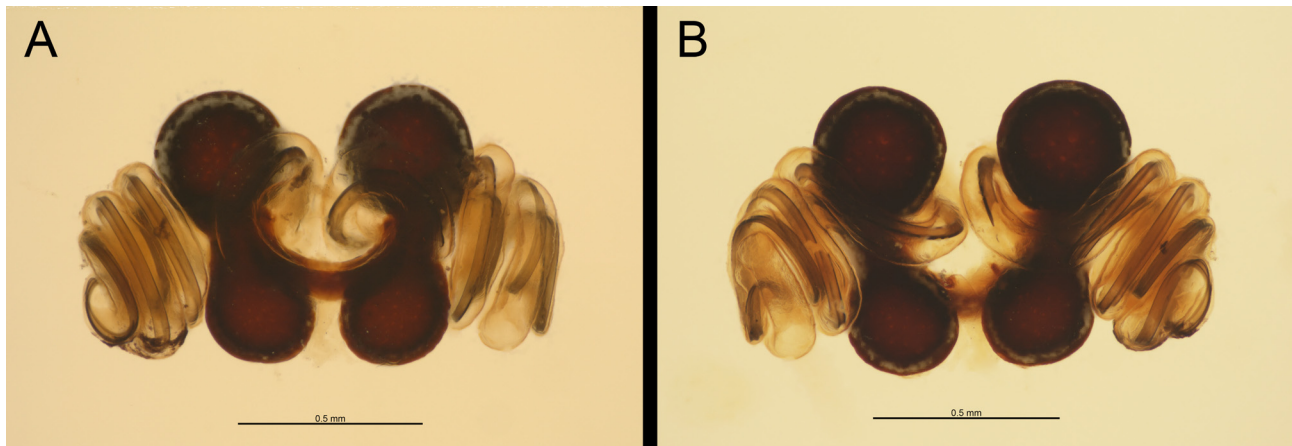


FIGURE 5. *Latrodectus umbukwane* n. sp. female. Images of reproductive organs with parallel spermathecae and looped copulatory ducts of paratype (NMSA29324). The copulatory ducts include the detached embolus of a mature male. A: epigynum, ventral view. B: vulva, dorsal view. Scale bars 0.5mm.

Discussion

The validity of *Latrodectus umbukwane* sp. n. is supported by the morphological and molecular evidence presented here. The discovery of this undescribed species of *Latrodectus* is surprising given the large size and striking appearance of the female, the medical importance of the genus, the taxonomic attention *Latrodectus* has received in southern Africa, and the relatively extensive survey effort that spiders have received in South Africa under the impetus of the South African National Survey of Arachnida (Dippenaar-Schoeman *et al.* 2015). This discovery suggests that the diversity of this genus may be underestimated in Africa, especially if there are other species which exhibit a similar degree of habitat specificity on other parts of the continent.

In gathering morphological data for *Latrodectus* from the literature, it was apparent that several species are incompletely described and have not received attention since their original descriptions. Again, surprisingly, it appears that the genus as a whole is in need of a global, detailed taxonomic revision. Both Kaston (1970) and Garb *et al.* (2004) suggested that genitalic characters are polymorphic in this genus and should be used with caution for delimiting species. However, phylogenetic analyses and divergent pairwise distances support the designation of *L. umbukwane* n. sp. as a new species. We suggest that in addition to natural history and morphology, multiple gene loci, including nuclear loci, should be considered over single locus approaches for automated species identification (Will *et al.* 2005; Dupois *et al.* 2012; Dowton *et al.* 2014; Liu *et al.* 2017; Yang & Rannala 2017). Coloration is clearly diagnostic for this new species, as it is in some other *Latrodectus* species. We support a view that species delimitation in *Latrodectus* should therefore include coloration, together with morphological, ecological and behavioral data in an integrated taxonomic framework.

TABLE 1. Summary of selected characteristics of females of African *Latrodectus* spp. from Dahl 1902, Schmidt *et al* 1994, Lotz 1994, Levy 1998 and Mirshamsi 2005. Abbreviations: TL = total length, CW = carapace width, T1 = tibia I length. * = data unavailable. Length measurements in mm.

	<i>L. umbukwane</i> n. sp.	<i>L. cinctus</i>	<i>L. indistinctus</i>	<i>L. karrooensis</i>	<i>L. renivulvatus</i>
TL	16.25 (n1); 14.00 (n2)	9.80 (7.25–12.45; n=10)	13.22 (11.25 –15.60; n=10)	11.09 (9.50–12.45; n=10)	10.37 (9.30–11.60; n=10)
CW	5.60 (n1); 6.00 (n2)	3.49 (3.10–4.20. n=10)	4.49 (4.10–5.00; n=10)	3.38 (3.50–4.50; n=10)	4.02 (3.35–4.45; n=10)
T1	9.00 (n1); 8.60(n2)	4.12 (3.55–4.90. n=10)	4.69 (4.50–5.00; n=10)	5.28 (4.30–6.20. n=10)	4.68 (4.30–5.30; n=10)
Spermathecae	parallel	V	V	V	V
Copulatory ducts	3 loops	3 loops	3 loops	3 loops	4 loops
Abdomen: Dorsal	Black, with white irregular circles dorsally, white outlined irregular transverse oblongs laterally, and with a red dorsal posterior stripe	Black with red dorsal posterior stripe. Sometimes 2–3 red transverse bands antero-dorsally	Similar to <i>L. cinctus</i>	Black with red T-shaped marking on dorsum	Dark brown to black, or black with transverse longitudinal orange-red bands. Highly variable
Abdomen: Ventral	Distinct red ventral marking anterior to spinnerets	Black	Black	Black with indistinct light yellow mark near epigastric furrow	Black
Dorsal abdominal setae	One type, uniform thick arched setae	Two types, medium long-thick and short thick setae	Two types, medium long-thick and short thick spines	Two types, medium long-thick and short thick spines	Two types, medium long-thick and short thick spines
Legs	Black	Black	Black	Black	Black
Egg Sac	Very large, smooth, purple-grey	Large, smooth, white-cream	Large, smooth, white-cream	Large, smooth, white-cream	Large, smooth, white-cream

.....continued on the next page

TABLE 1. (Continued)

	<i>L. renivulvatus</i>	<i>L. tredecimguttatus</i>	<i>L. geometricus</i>	<i>L. rhodesiensis</i>	<i>L. pallidus</i>
TL	10.37 (9.30–11.60; n=10)	11.88 (11.00–13.05; n=4)	10.84 (8.20–13.90; n=10)	10.35 (8.85–12.85; n=10)	11.00–13.00
CW	4.02 (3.35–4.45; n=10)	4.05 (3.60–4.60; n=4)	3.47 (2.50–4.30; n=10)	3.29 (2.65–3.70; n=10)	*
T1	4.68 (4.30–5.30; n=10)	4.68 (4.55–4.85; n=4)	5.60 (4.45–7.00; n=10)	5.35 (4.80–5.70; n=10)	*
Spermathecae	V	V	parallel	parallel	V
Copulatory ducts	4 loops	4 loops	3 loops	5 loops	3 loops
Abdomen: Dorsal	Dark brown to black, or black with transverse longitudinal orange-red bands. Highly variable	Black with 3 longitudinal series of red blotches dorsally; sometimes these blotches are absent and the opisthosoma is entirely black	Cream to black with loop-like brown blotches and 3 medial white-orange marks with dark center and borders	Similar to <i>L. geometricus</i> but blotches more distinctly outlined	Mostly white or off-white with dark brown spots in rows
Abdomen: Ventral	Black	Black and occasionally with a narrow red bar behind epigastric furrow	Distinct orange-red hour glass pattern	Distinct orange-red hour glass pattern	White to yellowish hourglass marking
Dorsal abdominal setae	Two types, medium long-thick and short thick spines	Two types, medium long-thick and short thick bifurcated setae	One type, two lengths of same fine hair-like setae; very fine very long setae	One type, two lengths of same fine hair-like setae	Widely dispersed (sparse) small spines
Legs	Black	Black	Banded	Banded	Banded
Egg Sac	Large, smooth, white-cream	Large, smooth, white-cream	Small, spiked, white-cream	Large, woolly, white-cream	Large, white-cream

TABLE 2. Summary of selected characteristics of males of African *Latrodectus* spp. from Dahl 1902, Schmidt *et al* 1994, Lotz 1994, Levy 1998, Mirshamsi 2005. Abbreviations: TL = total length, CW = carapace width, T1 = tibia I length. * = data unavailable. Length measurements in mm.

	<i>L. umbukwane</i> n. sp.	<i>L. cinctus</i>	<i>L. indistinctus</i>	<i>L. karrooensis</i>
TL	3.19 (2.75–3.50; n=7)	3.35 (3.00–3.85; n=10)	3.48 (2.60–4.15; n=10)	4.35 (3.93–4.85; n=10)
CW	1.09 (0.98–1.18) (n=6)	1.23 (1.05–1.35; n=10)	1.51 (1.25–1.80; n=10)	1.70 (1.65–1.75; n=10)
T1	2.08 (1.95–2.25; n=7)	2.88 (2.20–3.50; n=10)	2.93 (2.45–3.65; n=10)	3.75 (3.55–3.95; n=10)
Embolus	4 loops	3 loops	3 loops	3 loops
Abdomen: dorsal	At maturity complete white spots that fade with age	Three orange transverse bands (with indistinct centers on posterior pair)	Similar to <i>L. geometricus</i> but without dark spots and distinct posterior median stripe	Red T-shaped marking on dorsum as female but more brightly coloured
Abdomen: ventral	Yellow-white transverse ventral marking near the book lungs	2 orange transverse bands posterior with indistinct oblong mark in-between	Distinct orange-red hour glass pattern	As in female but more brightly coloured

TABLE 2. (Continued)

	<i>L. renivulvatus</i>	<i>L. tredecimguttatus</i>	<i>L. geometricus</i>	<i>L. rhodesiensis</i>	<i>L. pallidus</i>
TL	3.19 (2.55–4.00; n=10)	4.24 (4.10–4.40; n=4)	2.94 (2.65–3.25; n=10)	3.09 (2.60–3.75; n=10)	3.5–5.5
CW	1.16 (1.00–1.30; n=10)	1.60 (1.45–1.65)	1.02 (0.90–1.02; n=10)	1.17 (1.00–1.35; n=10)	*
T1	2.52 (2.10–2.90; n=10)	3.35 (3.00–3.60; n=4)	2.18 (1.85–2.35; n=10)	2.44 (2.00–2.85; n=10)	*
Embolus	4 loops	3 loops	4 loops	5 loops	3 loops
Abdomen: dorsal	3 continuous transverse orange-red bands and 3 longitudinal posterior short bands on a dark background	Anterior red transverse mark with 3 pairs of red lateral marks and 4 medial red marks	Broken light transverse areas on dark background	Similar to <i>L. geometricus</i>	Mostly white or off-white with dark brown spots in rows
Abdomen: ventral	Black	Narrow anterior red band discontinued in the middle and short posterior bar in front of spinnerets	Distinct orange-red hour glass pattern	Distinct orange-red hour glass pattern	White to yellowish hour-glass marking

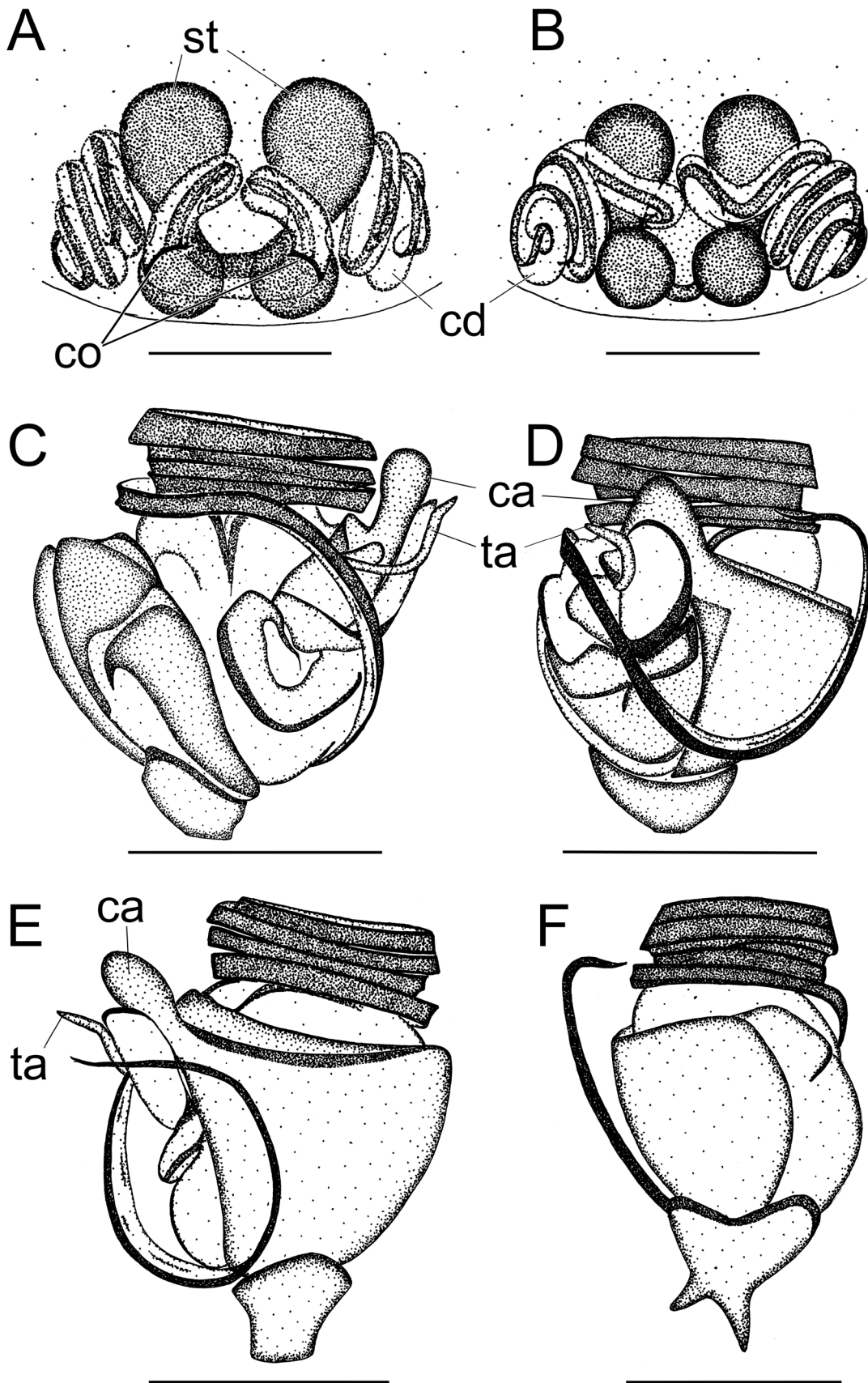


FIGURE 6. *Latrodectus umbukwane* n. sp. Illustrations of reproductive organs. A–B: female epigynum (A), ventral view, and vulva (B), dorsal view. C–F: sinistral male pedipalp ventral view (C), retrolateral view (D), prolateral view (E) and dorsal view (F). ca: cymbial apophysis; cd: copulatory ducts; co: copulatory openings; st: spermathecae; ta: tegular apophysis. Scale bar = 0.5mm.



FIGURE 7. *Latrodectus umbukwane* n. sp. immatures. A: second instar spiderlings on emergence from the egg case; B: third instar spiderling; C: fourth instar showing white dorsal markings; D: larger immature female showing distinctive yellow and white markings dorsally and laterally.

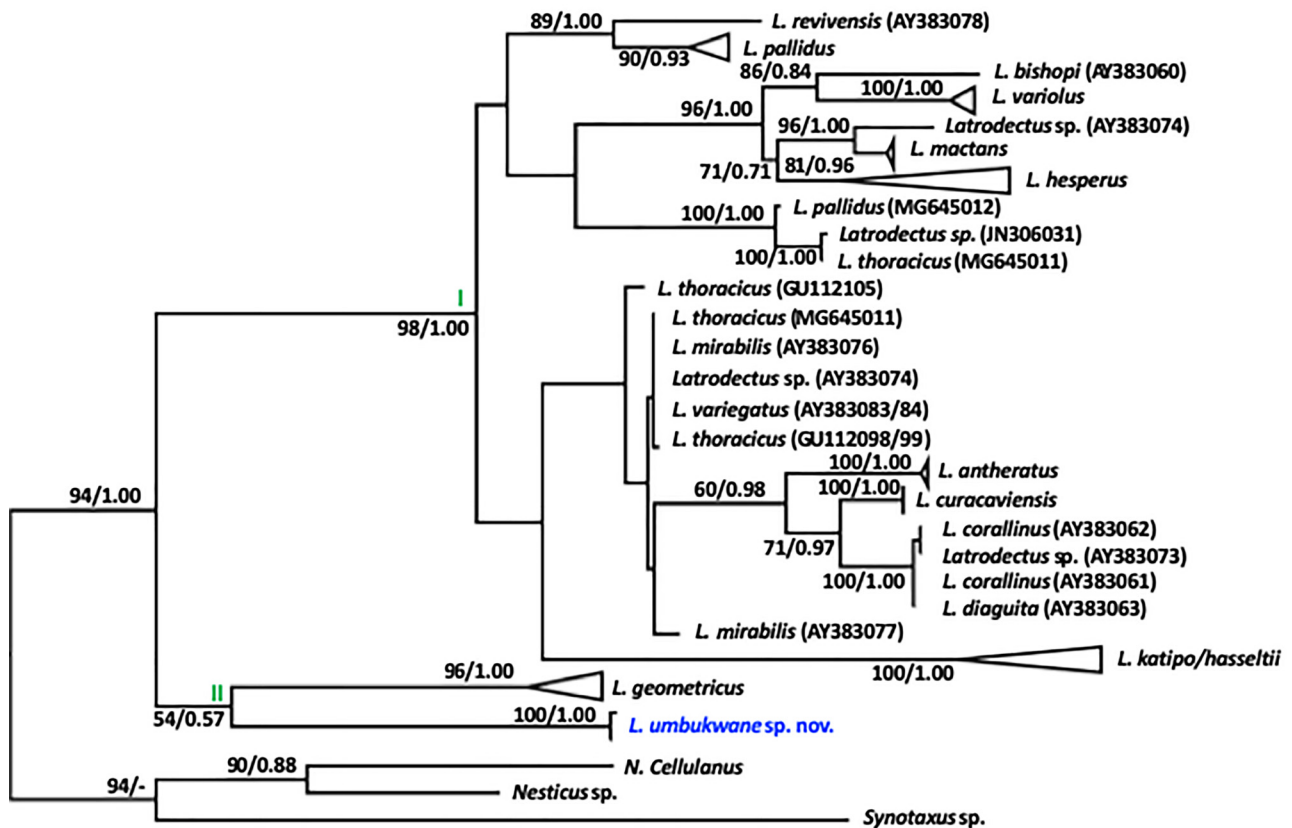


FIGURE 8. Maximum Likelihood phylogram with bootstrap and posterior probability support overlaid. Values in brackets are GenBank accession numbers or BoLD identifiers. Where more than one individual from a species was included only the species name appears on the phylogram. The GenBank accession number/BoLD identifier can be found in Supplementary Material Table S1.

Acknowledgements

We wish to thank the staff of &Beyond Phinda Private Game Reserve for their support of the project. Jason Dunlop and Anja Friederichs of the Museum für Naturkunde Berlin assisted with images of type specimens of *Latrodectus obscurior*. We would also like to thank Richard and Naomi Schutte for alerting us to the first specimen, Simon and Tamsin Naylor for discovering the second specimen, Ross Goode for locating additional specimens, and Richard Wright for helping to feed thousands of spiderlings. Petro Marais, Aisha Mayekiso, Kirstin Williams and Matabaro Ziganira assisted with accessioning specimens and providing catalog numbers. Sizwe Dladla proposed the iSiZulu name. Raesetsa Mailula prepared the SEM images. We thank Jeremy Miller, Gustavo Hormiga and an anonymous reviewer for their comments on an earlier version of this manuscript. We especially acknowledge Wild Tomorrow Fund for funding this work. Catherine Sole is funded by the National Research Foundation.

Key to the southern African species of the genus *Latrodectus* (adapted from Lotz 1994)

- 1 Females 2
- Males 8
- 2 Spermathecae parallel, abdomen with red or orange-red marking on ventrum 3
- Spermathecae in a V-formation, abdomen without red or orange-red hourglass marking on ventrum 5
- 3 Ventral marking is orange-red and hour glass shaped 4
- Ventral marking is red and an irregular oblong shape *L. umbukwane n. sp.*
- 4 Spermathecal ducts with 3 loops *L. geometricus*
- Spermathecal ducts with 5 loops *L. rhodesiensis*

5	Abdomen with red T-shaped marking on dorsum	<i>L. karrooensis</i>
-	Abdomen without red T-shaped marking on dorsum	6
6	Spermathecal ducts with 4 loops	<i>L. renivulvatus</i>
-	Spermathecal ducts with 3 loops	7
7	Carapace width less than 3.8 mm	<i>L. cinctus</i>
-	Carapace width more than 3.8 mm	<i>L. indistinctus</i>
8	Embolus with 5 loops	<i>L. rhodesiensis</i>
-	Embolus with 4 or less loops	9
9	Embolus with 4 loops	10
-	Embolus with 3 loops	12
10	No light transverse areas on abdomen and an oblong yellow-white ventral marking caudally and white oblong near book lungs, abdomen brown with light yellow-white oblong blotches dorsally and laterally outlined in darker brown, no transverse light areas (young mature male), or abdomen dark brown with darker indistinct irregular circles dorsally and ventrally (old mature male)	<i>L. umbukwane</i> n. sp.
-	Abdomen dorsally with light transverse areas on dark background	11
11	Abdomen dorsally with broken light transverse areas on a darker background	<i>L. geometricus</i>
-	Abdomen dorsally with continuous light transverse areas on a dark background	<i>L. renivulvatus</i>
12	Abdomen dark with dorsally an orange-red T-shaped marking	<i>L. karrooensis</i>
-	Dorsal abdominal pattern without T-shaped marking	13
13	Abdomen with broken light dorsal transverse areas connected with indistinct light areas, anterior light transverse area continuous	<i>L. cinctus</i>
-	Broken light dorsal transverse areas unconnected	<i>L. indistinctus</i>

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