Molecular and morphological species delimitation suggest a single species of the beetle-spider genus Ballus in Sri Lanka (Araneae: Salticidae)

Authors: Bopearachchi, Dilini P., Eberle, Jonas, and Benjamin, Suresh P.

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Molecular and morphological species delimitation suggest a single species of the beetle-spider genus *Ballus* in Sri Lanka (Araneae: Salticidae)

Dilini P. Bopearachchi[1], **Jonas Eberle**[2] and **Suresh P. Benjamin**[1]: [1]National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka. E-mail: suresh.benjamin@gmail.com; [2]Evolutionary Zoology, University of Salzburg, Hellbrunner Straße 34, 5020 Salzburg, Austria

Abstract. *Ballus* Koch, 1850 is a beetle-like jumping spider genus encountered in montane evergreen rainforests of the Central and Uva Provinces of Sri Lanka. The taxonomic literature documents three species of the genus for the island. However, neither the taxonomic validity nor the systematics of any of the three species have been previously examined. We used nuclear and mitochondrial DNA sequences (*28S* rRNA, *H3*, *COI*) as well as morphological characters to investigate the genetic and taxonomic diversity of *Ballus* populations in Sri Lanka, including specimens from type localities. No *Ballus* specimens were found outside of the central highlands. Results of molecular species delimitation and morphological analysis suggest the presence of only a single species of *Ballus* in Sri Lanka. We therefore propose *B. sellatus* Simon, 1900 to be a junior synonym of *B. segmentatus* Simon, 1900, while *B. clathratus* Simon, 1901 remains a *nomen nudum*. Further, we discuss the implications of our results for conservation planning.

Keywords: Morphology, arachnid, DNA barcoding, species discovery, island biogeography

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Spiders are one of the most diverse groups of invertebrates in the world. The family Salticidae (jumping spiders), including 6359 described species in 659 genera and seven subfamilies, is the largest spider family comprising about 13% of all known diversity (Maddison 2015; World Spider Catalog 2021). Salticids are also the most diverse spider family in Sri Lanka (Benjamin et al. 2012). The genus *Ballus* Koch, 1850 currently comprises nine described species of which most occur in the Mediterranean region (World Spider Catalog 2021): *B. armadillo* (Simon, 1871), *B. chalybeius* (Walckenaer, 1802), *B. japonicas* (Saito, 1939), *B. piger* (O. Pickard-Cambridge, 1876), *B. rufipes* (Simon, 1868), *B. segmentatus* (Simon, 1900), *B. sellatus* (Simon, 1900), *B. tabupumensis* (Petrunkevitch, 1914), and *B. variegatus* (Simon, 1876). The type species is *B. chalybeius*, with a distribution from North Africa and Europe to Central Asia (World Spider Catalog 2021).

Within Sri Lanka, the genus *Ballus* is known from the island's central montane wet evergreen forests. *Ballus segmentatus* and *B. sellatus*, the two valid species from the island mentioned in the literature, were originally described in the same publication by Simon (1900). In 1901, Simon also listed a third Sri Lankan species, *B. clathratus* Simon, 1901 (Simon 1901). However, he never formally described it and it was subsequently designated a *nomen nudum* by Bonnet (1955). *Ballus segmentatus* (Fig. 1) has since been re-described based on freshly collected material (Benjamin 2004).

Sri Lanka's biodiversity is increasingly threatened by human activities, such as intrusion and disturbance of remnant mountain forest, cultivation of European vegetable varieties, poaching and collection of forest products, climate change and severe weather, biological invasions, and modification of forests for hydropower generation (Benjamin & Kanesharatnam 2016). The genus *Ballus* in Sri Lanka is known from relatively few individuals, and all localities are restricted to mid- or high-altitude cloud forest (900–1800 m above sea level) in the Central and Uva Provinces. Our decades-long sampling has resulted in only 28 *Ballus* specimens, raising concerns about their conservation status. However, the group's ambiguous taxonomy makes it difficult to accurately determine threats and assess the species' conservation needs.

In this study, we assess the number and distribution of *Ballus* species in Sri Lanka, based on morphological data as well as mitochondrial and nuclear DNA markers, following an island-wide sampling regime. Further, we revise the taxonomy of Sri Lankan *Ballus* species and discuss the implications of our findings for the conservation of the genus.

METHODS

Specimen collection.—This study is based on a total of 28 *Ballus* specimens collected since 1996. Fieldwork was conducted in all climatic regions of Sri Lanka sampling over 100 sites across the island. However, *Ballus* specimens were only found at eight localities in the central highlands in habitats above 900 m. The spiders were collected by beating vegetation and general hand collection. Seven specimens were preserved in 70% ethanol for morphological investigation and 21 specimens were preserved in absolute ethanol for molecular analysis. At least two individuals from each locality were included whenever possible. Outgroup selection of five taxa relied on published studies on the molecular phylogeny of Salticidae (Maddison 2015) and on the morphological (Benjamin 2004) and molecular phylogeny of Ballinae (Bopearachchi 2020).

Taxonomic methods.—Specimens preserved in 70% ethanol were examined using a Leica S9E binocular stereomicroscope (Leica Microsystems Limited, Wetzlar, Germany). Left male palps were dissected and immersed in methyl salicylate, slide mounted, observed and illustrated with the aid of Leica DM3000 LED stereo microscope with an attached drawing tube. The female epigastric region was dissected and digested in a pancreatin solution (Alvarez-Padilla & Hormiga 2007) for

Figure 1.—Photographs of *Ballus segmentatus* in life. Images by Suresh P. Benjamin.

ca. 3–7 days, slide-mounted and illustrated as above. All measurements are in millimetres. Body length was measured as carapace length plus abdomen length (excluding the spinnerets). Types and other specimens were borrowed from the Natural History Museum, Paris (MNHN). All studied specimens are deposited in the National Museum of Sri Lanka, Colombo.

Molecular analysis.—Three gene regions were selected for polymerase chain reaction (PCR) amplification: the protein-coding mitochondrial gene cytochrome *c* oxidase subunit I (*COI*); the nuclear large subunit ribosomal RNA gene (*28S*); and the protein-coding nuclear gene histone *H3* (*H3*). *COI* is more suitable to resolve more recent evolutionary events, whereas *28S* and *H3* resolve deeper nodes in phylogenetic trees (Edgecombe & Giribet 2006). *COI* has furthermore been used as a standard DNA barcode region for many years (Costa et al. 2007). Total genomic DNA extraction, PCR and sequencing were done according to Kanesharatnam & Benjamin (2019). Accession numbers for all sequences and locality information are provided in Table 1. Details of PCR primers,

Table 1.—Details of exemplars used in this study including collection localities, GenBank accession and NIFS voucher numbers. Accession numbers in **bold** denote sequences newly generated for this study. All species belong to the family Salticidae. NIFS_Sal_723 was not included in the phylogenetic analysis. CP: Central Province; SL: Sri Lanka; UV: Uva Province.

Species	Locality	*28S*	*H3*	*CO1*	Catalogue number
Ingroup taxa					
Ballus segmentatus	SL, CP, Riverston	_	**MT828412**	**MT828376**	NIFS_Sal_051
Ballus segmentatus	SL, UP, Namunukula	_	**MT828413**	**MT828377**	NIFS_Sal_053
Ballus segmentatus	SL, CP, Samimale	**MT828366**	**MT828414**	**MT828378**	NIFS_Sal_057
Ballus segmentatus	SL, CP, Hakgala	_	**MT828415**	**MT827379**	NIFS_Sal_263
Ballus segmentatus	SL, CP, Horton Plains	**MT828367**	**MT828416**	**MT828380**	NIFS_Sal_477
Ballus segmentatus	SL, CP, Horton Plains	**MT828368**	**MT828417**	**MT828381**	NIFS_Sal_478
Ballus segmentatus	SL, CP, Upcot	**MT828369**	**MT828418**	**MT828382**	NIFS_Sal_487
Ballus segmentatus	SL, CP, Loolkandura	**MT828370**	**MT828419**	_	NIFS_Sal_525
Ballus segmentatus	SL, CP, Agrabopath	**MT828371**	**MT828420**	_	NIFS_Sal_527
Ballus segmentatus	SL, CP, Horton Plains	**MT828372**	**MT828421**	**MT828383**	NIFS_Sal_528
Ballus segmentatus	SL, UP, Namunukula	_	**MT828422**	_	NIFS_Sal_723
Ballus segmentatus	SL, CP, Hakgala	_	**MT828423**	**MT828384**	NIFS_Sal_850
Ballus segmentatus	SL, CP, Hakgala	**MT828373**	**MT828424**	**MT828385**	NIFS_Sal_851
Ballus segmentatus	SL, CP, Horton Plains	_	_	**MT828386**	NIFS_Sal_1064
Ballus segmentatus	SL, CP, Loolkandura	_	**MT828425**	**MT828387**	NIFS_Sal_1069
Ballus segmentatus	SL, CP, Piduruthalagala	**MT828374**	**MT828426**	**MT828388**	NIFS_Sal_1081
Ballus segmentatus	SL, UP, Ohiya	**MT828375**	**MT828427**	_	NIFS_Sal_1189
Ballus chalybeius	Germany	EF514398	_	EF514383	
Ballus chalybeius	Germany	_	_	KX537317	
Ballus chalybeius	Germany	_	_	KY268728	
Ballus chalybeius	Germany	_	_	KY269663	
Ballus chalybeius	Germany	_	_	KY269570	
Peplometus sp.	Ghana	EU815515	_	EU815621	
Padilla mitohy	Madagascar	EF514377	_	EF514392	
Leikung sp.	Malaysia	_	KY018404	KY017895	
Afromarengo sp.	Gabon	JX145758	_	JX145682	
Mantisatta longicauda	Philippines	AY297270	_	AY297399	

their sequences, annealing temperatures and related references are provided in Table 2.

Phylogenetic and species delimitation analyses.—Sequences were assembled and edited using the Geneious 11.0.2 software package (Biomatters Limited; Kearse et al. 2012). Sequence alignment was conducted separately per gene with MAFFT (v. 7.450; Katoh & Standley 2013), using the high accuracy G-INS-I settings. Multiple sequence alignments were checked by eye in Aliview (v. 1.26; Larsson 2014) for obvious errors like internal stop codons or reading frame shifts. Furthermore, both ends of alignments with more than 50% missing data were discarded prior to downstream analyses. Additional *COI*, *28S* and *H3* sequences of Salticidae were obtained as outgroup taxa from GenBank (Table 1).

A phylogenetic tree based on the three concatenated genes was inferred using IQ-TREE (v. 1.6.12; Nguyen et al. 2015). Three initial partitions, one per gene, were subjected to the implemented Modelfinder (Kalyaanamoorthy et al. 2017) to infer an optimal partition scheme and substitution models. Branch support was estimated by 1000 Ultrafast Bootstrap (UFB) (Hoang et al. 2018) replications. The tree with the highest likelihood out of 10 independent replicate runs was chosen and rooted with *Afromarengo* sp. in Dendroscope (v. 3.7.2; Huson & Scornavacca 2012); support values from

Table 2.—Gene targets, PCR conditions and nucleotide sequences of the primers used in this study. References: a, Hedin and Maddison (2001); b, Folmer et al. (1994); c, Spagna and Gillespie (2008); d, Colgan et al. (1998).

Target gene	Annealing temperature/time	Primer pair	5′ -Primer sequence- 3′	Reference
CO1	47°C/ 1.3 min	C1-J-1718 (F)	GGA GGA TTT GGA AAT TGA TTA	a
		C1-N-2191 (R)	CCC GGT AAA ATT AAA ATA TAA ACT TC	a
	48°C/ 50s	LCO-1628 (F)	ATA ATG TAA TTG TTA CTG CTC ATG C	a
		C1-N-2191	CCC GGT AAA ATT AAA ATA TAA ACT TC	a
	46°C/50s	LCO -1490(F)	GGT CAA CAA ATC ATA AAG ATA TTG G	b
		C1-N-2191 (R)	CCC GGT AAA ATT AAA ATA TAA ACT TC	b
28S	55°C/ 45s	28S O (F)	GAA ACT GTC CAA AGG TAA ACG G	a
		28S C (R)	GGT TCG ATT AGT CTT TCG CC	c
H3	51°C/ 45s	H3a (F)	ATG GCT CGT ACC AAG CAG ACV GC	d
		H3a (R)	ATA TCC TTR GGC ATR ATR GTG AC	d

bootstrapping were treated as branch supports as opposed to node supports. A gene tree for *COI* was inferred the same way.

We employed three commonly used algorithms for *de novo* species delimitation which are based on different assumptions. First, the tree-based multi-rate Poisson Tree Process (mPTP; Linux version 0.2.4; Kapli et al. 2017), which requires monophyly of species and models the Yule-coalescent transition points based on the change of substitution rates on the phylogenetic input tree (Zhang et al. 2013); and second, a distance-based clustering approach, Automatic Barcode Gap Detection (ABGD; version from December 2011; Puillandre et al. 2012), which relies on the assumption of a barcode gap being present; and third, BPP (v. 4.3.0; Yang 2015) to perform Bayesian multi-locus species delimitation based on the multispecies coalescent model.

The *COI* gene tree (see above) was used as the input tree for the mPTP analysis, for which the program's MCMC algorithm was used to estimate delimitation supports for the maximum likelihood estimate of the species model. Multiple rates were allowed for putative species. The chain ran for 10 million generations, sampling every 200,000 generations. One million generations were discarded as burnin. For ABGD, we used the substitution model-based maximum likelihood estimate of pairwise *COI* distances from the IQ-TREE analysis (see above) as input. The minimum prior intraspecific distance was set to 0.1%, the maximum to 10%. Ten steps were tested in between. The minimum slope increase (X) was left at the default of 1.5.

BPP uses the multispecies coalescent model to compare different species models in a Bayesian framework (Yang & Rannala 2010; Rannala & Yang 2013), however this method tends to over-split species (Sukumaran & Knowles 2017; Eberle et al. 2019; Chambers & Hillis 2020). Therefore, it is rather unlikely that species groups recognized by BPP mistakenly consist of multiple species. The topology of the species tree was previously estimated with IQ-TREE based on all three genes, as described above. Prior to the actual species delimitation analysis, we used BPP to obtain rough estimates of θ and τ_0. Such estimates can be used to inform prior choice in the final species delimitation analyses (Yang 2015). In this preliminary analysis, individuals were assigned to previously determined species based on morphology. Sixteen *Ballus* specimens from Sri Lanka were treated as one species. τ_0 was set so that its mean reflected approximately half the number of substituted bases between *Afromarengo* sp. and *B. segmentatus* (inverse gamma prior for $\tau_0 = \text{IG}\ [\alpha = 3, \beta = 0.035]$ with mean = 0.0175, meaning 1.75% of sequence divergence between the root and present time). In the final analyses, diffuse θ- and τ_0-priors ($\alpha = 3$) with means according to the before estimated values were used (θ: IG $[\alpha = 3, \beta = 0.4]$, mean = 0.2; and τ_0: IG $[\alpha = 3, \beta = 0.035]$, mean = 0.0175). Such uninformative prior distributions allow enough variability to sufficiently sample a potential split of *B. segmentatus* in the MCMC chain. Internal node heights were specified by the uniform Dirichlet distribution (Yang & Rannala 2010). In the final analysis, each individual was initially assigned to its own potential species cluster to allow splitting of previously determined species. After a burnin of 100,000 generations, the MCMC chain ran for 1 million generations, sampling every 100th generation. Each final analysis was run three times to confirm consistency between runs (see Supplemental Fig. S1, online at https://doi.org/10.1636/JoA-S-21-040.s1). The R package coda (Plummer et al. 2006) was used to assess convergence of runs after filtering the MCMC output files for the most frequently sampled species model with UNIX grep. ESS values were assured to be >200. Splits in the species tree were considered to represent a speciation event if posterior probabilities were > 0.95.

RESULTS AND DISCUSSION

Morphological taxonomy.—Simon (1900) described two species of *Ballus* from Sri Lanka. However, the type specimen of *B. segmentatus* is a male whereas that of *B. sellatus* is a female. We examined 28 Sri Lankan *Ballus* specimens using standard methods in salticid taxonomy. We found no characters that could be used to unambiguously diagnose more than one species of *Ballus* in Sri Lanka. Some variation in color pattern, markings and size was observed. However, this observed variation does not warrant the recognition of two or more species. Other studies (Alicata & Cantarella 1988; Benjamin 2004) also questioned the presence of two species in Sri Lanka. Alicata & Cantarella (1988) mentioned that Simon's (1900) descriptions of Sri Lankan *Ballus* species are very brief and that the type localities of the two species are identical.

As often in taxonomy, and in particular in arachnology, morphology plays a dominant role. Prior to the spread of Hennigian methods, most taxonomists based their classifications and evolutionary scenarios upon single character systems that were believed *a priori* to have greater importance than other characters (see Benjamin 2004 for a review). The classifications of *Ballus* species by Simon (1900) were based on character systems believed to be of superior importance at the time of study, such as body coloration or size, and the placement and coloration of setae. The descriptions of *B. sellatus* and *B. segmentatus* were likely based on poorly defined and highly variable characters.

Phylogenetic and species delimitation analyses.—Our matrix of the three concatenated mitochondrial and nuclear markers included 26 *Ballus* specimen terminals. This included 16 samples from Sri Lanka and five samples of *B. chalybeius* (the generic type species) from Germany. The total length of the final matrix was 1,525 bp. The lengths of the sequenced fragments excluding the primers were as follows: *H3*, 285 bp; *28S*, 783 bp; and *COI*, 685 bp. The monophyly of Sri Lankan *Ballus* was recovered and well supported (UFB = 100; Fig. 2). The low levels of support for many nodes within the *B. segmentatus* clade (UFB < 50) is caused by insufficient information in the available sequence data to discriminate between very recently diverged and admixing populations, also reflected in the relatively very short internal branch lengths within the same clade. These results thus suggest the presence of a single species of *Ballus* in Sri Lanka. Furthermore, the phylogeny recovered a sister-group relationship between *B. segmentatus* and *Peplometus* sp. and not between samples of European *B. chalybeius*, which implies that *Ballus*, as currently defined, is paraphyletic. This issue will be addressed in a future publication.

All of the molecular species delimitation analyses (ABGD, mPTP and BPP) we conducted supported the existence of a

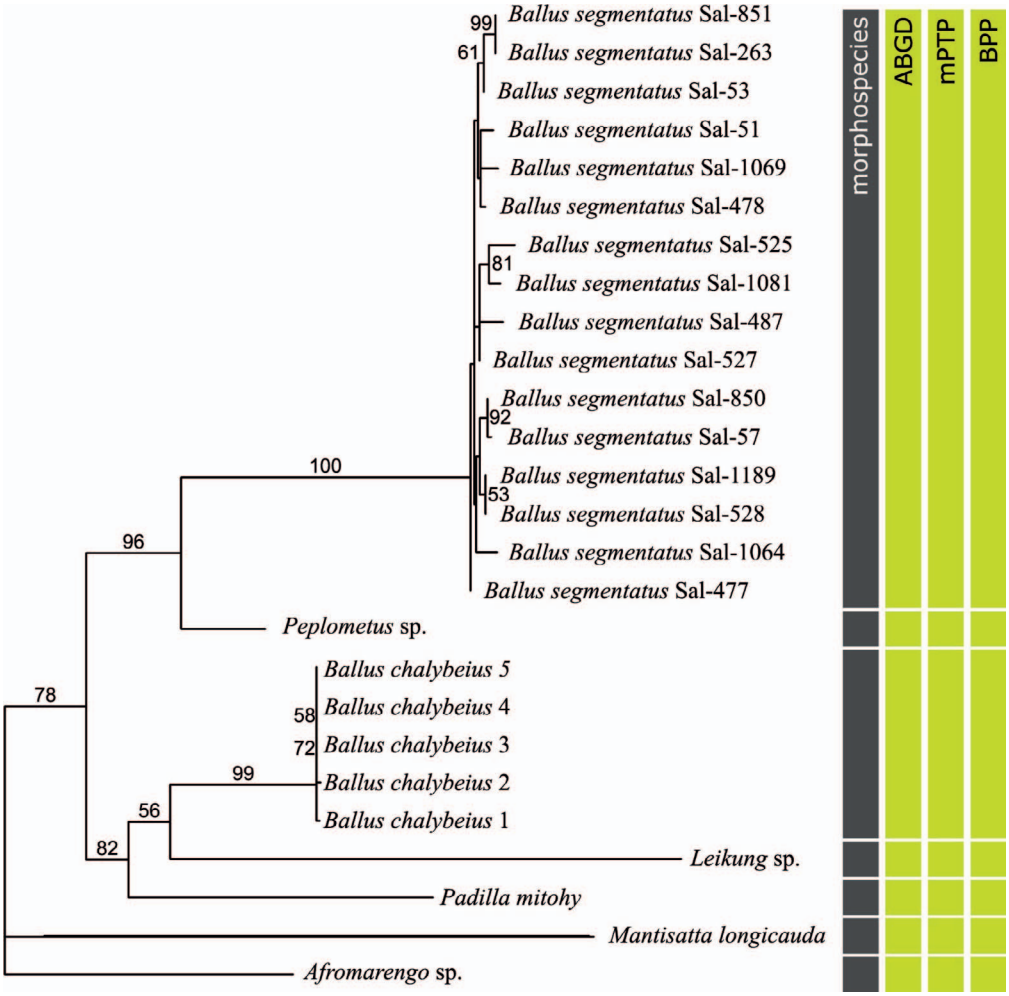

Figure 2.—Phylogenetic tree based on the concatenated partitioned matrix of three genes (*COI*, *28S* and *H3*), inferred with IQ-TREE. Numbers on branches show bootstrap values (values < 50 are not shown). Columns to the right of the tree indicate species recognised by morphology (morpho-species) and results of the three species delimitation analyses.

single Sri Lankan *Ballus* species (Fig. 2). mPTP inferred zero support of there being a speciation event for all internal nodes in both *Ballus* clades except the basal ones, which was close to zero as well (0.02 for Sri Lankan *Ballus* and 0.005 for *Ballus chalybeius*). ABGD inferred the same species for all initial intraspecific distances and in all initial and recursive partitions, clearly rejecting the presence of more than one species of *Ballus* in Sri Lanka. Like the *COI*-based mPTP analysis, multi-locus species delimitation with BPP found some genetic structure among Sri Lankan *Ballus* specimens, which was, however, insufficient to support distinct species. The two deepest nodes within the *B. segmentatus* clade, separating the individual Sal-477 and two internal clades, received mean posterior probabilities to be speciation events of 0.88 (0.87–0.88) and 0.75 (0.75–0.76), respectively.

Conclusion.—Our results lead us to propose the presence of only a single species of *Ballus* in Sri Lanka. The lack of unambiguous diagnostic characters in morphology, together with the lack of molecular evidence for separating the study samples into two or more species justifies synonymizing *B. sellatus* Simon, 1900 under *B. segmentatus* Simon, 1900.

TAXONOMY

Family Salticidae Blackwall, 1841
Genus *Ballus* C. L. Koch, 1850

Ballus C. L. Koch, 1850: 68.

Type species.—*Salticus heterophthalmus* Wider, 1834, synonym of *Ballus chalybeius* (Walckenaer, 1802).

Ballus segmentatus Simon, 1900

Ballus segmentatus Simon, 1900: 398; Simon, 1901: 482; Benjamin, 2004: 30.
Ballus sellatus Simon, 1900: 398. **New synonymy**.

Type material.—*Syntypes*: SRI LANKA: 2 ♂, 1 ♀, "Colombo, Kandy" (MNHN: 20407/2297), examined. The ♀ syntype of *B. sellatus*, which probably should have been in MNHN was not found and is presumed lost. There was also no catalogue card for this species. However, a second vial of several specimens of *Ballus* from Sri Lanka was found in the

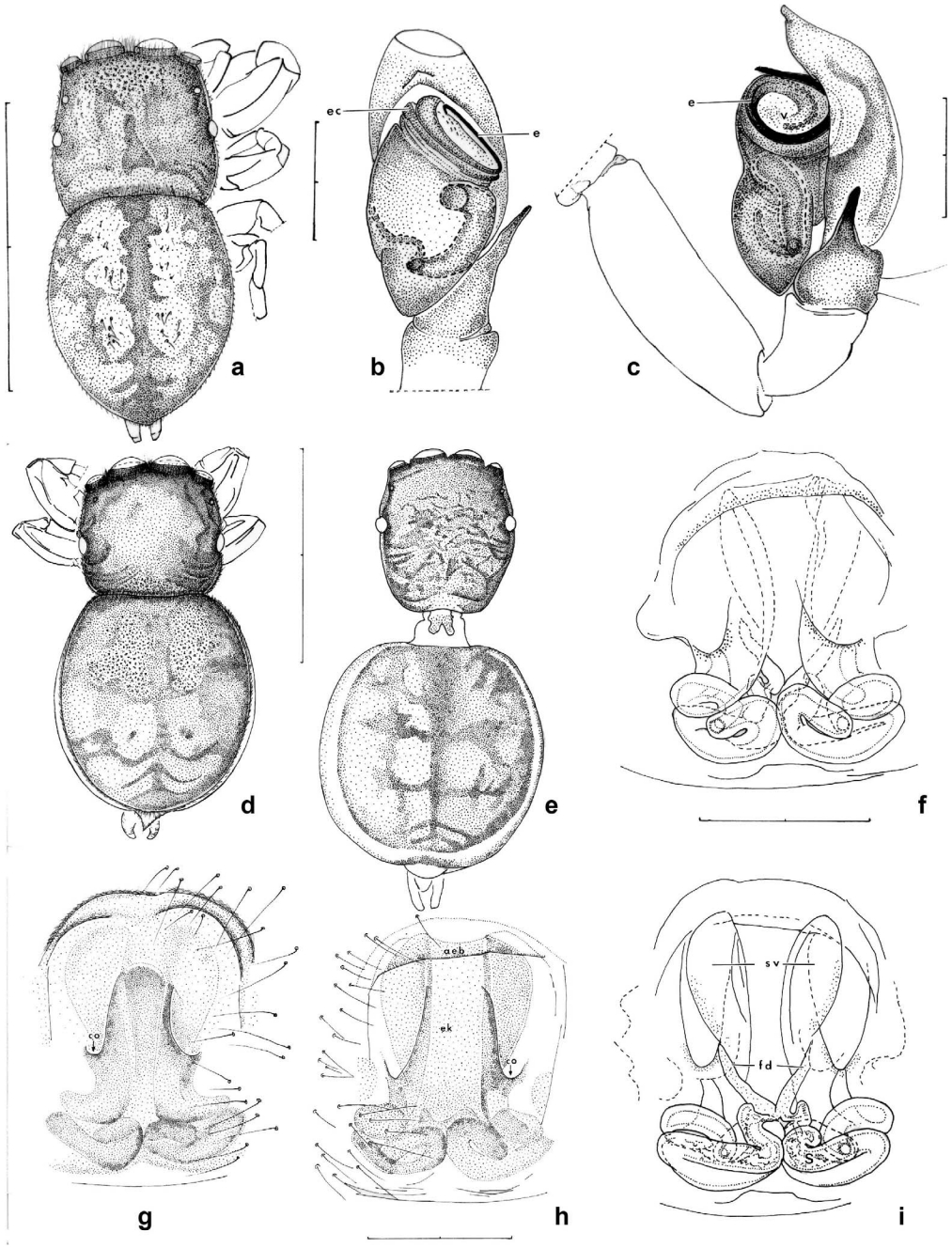

Figure 3.—*Ballus segmentatus* Simon, 1900. a–d, g, Male and female from Agrapatana, Sri Lanka: a, male habitus, dorsal view; b, male palp, ventral view; c, same, retrolateral view; d, female habitus, dorsal view. e–i, Female from Hakgala: e, habitus, dorsal view; g, h, epigynum, ventral view; f, vulva, ventral view; i, same, dorsal view. Scale bars = 0.2 mm (b, c, f–i); 2.0 mm (a, d, e).

same bottle (#2297). These specimens are indistinguishable from the syntypes.

It is possible that the ♀ syntype of *B. sellatus* was incorporated by mistake into either of the two vials mentioned above. The curator in charge of the material confirms that such mixing and relabelling might have taken place.

Other material examined.—SRI LANKA: *Central Province*: 1 ♂, Nuwara Eliya District, Horton Plains National Park, 06°48′04″N, 80°48′23″E, alt. 2130 m., 20–21 February 2007, S.P. Benjamin & Z. Jaleel (NIFS_SAL_062); 1 ♂, same data (NIFS_SAL_528); 1 ♀, Agarapatana-Bopathalawa Forest Reserve, bordering Torrington Estate, near Agarapatana, 06°50′36″N, 80°40′40″E, alt. 1621 m., 18–21 February 2007, S.P. Benjamin & Z. Jaleel (NIFS_SAL_527); 1 ♂, Upcot, 06°46′N, 80°36′E, alt. 1850 m., 2 February 2011, S.P. Benjamin & S. Batuwita (NIFS_SAL_057); 1 ♀, Upcot, 06°46′N, 80°36′E, alt. 1848 m., 14 February 2012, S.P. Benjamin & N. Athukorala (NIFS_SAL_487); 2 ♂, Horton Plains National Park, 06°48′04″N, 80°48′23″E, alt. 2130 m., 27 March 2012, S.P. Benjamin et al. (NIFS_SAL_477–478); 1 ♀,

Hakgala Strict Nature Reserve, 06°55′52″N, 80°48′46″E, alt. 1768 m., 20 January 2015, N. Athukorala et al. (NIFS_SAL_263); 1 ♂, 2 ♀, same locality data, 06°55′44″N, 80°48′55″E, alt. 1082 m., 30 June 2016, N. Athukorala et al. (NIFS_SAL_850–852); 3 ♀, Horton Plains National Park, 06°47′54″N, 80°48′51″E, alt. 2136 m., 22 June 2017, N. Athukorala et al. (NIFS_SAL_1064-1066); 1 ♂, 1 ♀, 1 juvenile, Piduruthalagala, near 5 km post, 06°59′56″N, 80°46′33″E, alt. 2437 m.,14 February 2018, S.P. Benjamin et al. (NIFS_SAL_1079-1081); 2 juveniles, Horton Plains National Park, 06°47′55″N, 80°47′39″E, alt. 2146 m., 30 January 2019, S.P. Benjamin et al. (NIFS_SAL_1193-1194); 1 ♀, same locality data, Dayagama trail, 06°48′46″N, 80°47′53″E, alt. 2086 m., 3 July 2019, S.P. Benjamin et al. (NIFS_SAL_1226); 1 ♂, Kandy District, Delthota, Forest adjacent to Loolkandura estate, 07°08′44″N, 80°42′02″E, alt. 1480 m., 11 May 2010, S. Batuwita & N. Athukorala (NIFS_SAL_525); 1 ♂, same locality data, 25 January 2011, S.P. Benjamin & S. Batuwita (NIFS_SAL_048); 2 ♀, same locality data, 15 November 2017, N. Athukorala et al. (NIFS_SAL_1069-1070); 1 ♂, Matale District, Riverston 07°31′18″N, 80°44′10″E, alt. 1259 m., 21 June 2012, S.P. Benjamin et al. (NIFS_SAL_051). *Uva Province*: 1 ♂, Badulla District, Ohiya, 06°50′32″N, 80°53′05″E, alt. 1288 m., 30 August 2011, S.P. Benjamin & N. Athukorala (NIFS_SAL_1189); 1 ♀, same locality data, 26 May 2012, N.P. Athukorala (NIFS_SAL_419); 1 ♀, along Passara Ella Road (B113), Namunukula, 06°52′N, 81°07′E, alt. 1313 m., 23 January 2014, S.P. Benjamin & N. Athukorala (NIFS_SAL_723); 1 ♂, same locality data, litter, 27 February 2015, same collectors (NIFS_SAL_053).

Diagnosis.—Males are separated from European *Ballus* spp. by the presence of a pseudo-conductor, reticulate markings on the opisthosoma, and tapering retrolateral tibial apophysis (RTA) (Figs. 3B, C). The female can be distinguished by the absence of an enlarged, sac-like translucent septum (Figs. 3F–I; see also Benjamin, 2004: figs. 21a, c, 22a, c, 24g, h, 23a, b).

Description (male).—Prosoma rounded, dark brown. Opisthosoma rounded, with reticulate markings (Figs. 1A–I). Total length 3.6: prosoma length 1.4, width 1.3; opisthosoma length 1.5, width 1.4. Eyes surrounded by dark rings. Eye diameters, inter-distances, and median ocular quadrangle: AME 0.4; ALE 0.22; PME 0.08; PLE 0.2; PLE–PLE 1.24; PME–PME 0.8; ALE–PME 0.54; ALE–PLE 0.22. Legs light yellow, laterally without dark markings. Leg measurements: I: 2.4 (0.6, 0.42, 0.63, 0.48, 0.27); II: 1.77 (0.48, 0.3, 0.45, 0.36, 0.18); III 1.74 (0.57, 0.24, 0.45, 0.3, 0.18); IV: 1.83 (0.63, 0.24, 0.51, 0.27, 0.18). Pedipalp with oval cymbium, RTA stout and tapering to a pointed end, tegulum bilobed, projecting outwards, embolus stout and coiled once (Figs. 3B, C).

Description (female).—Prosoma rounded, dark brown. Opisthosoma rounded, with reticulate markings (Figs. 1A–I). Total length 3.4: prosoma length 1.1, width 1.2; opisthosoma length 1.9, width 1.6. Eyes surrounded by dark rings. Eye diameters, inter-distances, and median ocular quadrangle: AME 0.5; ALE 0.24; PME 0.09; PLE 0.27; PLE–PLE 1.68; PME–PME 1.38; ALE–PME 0.6; ALE–PLE 0.3. Legs light yellow, laterally without dark markings. Leg measurements: I: 2.2 (0.66, 0.38, 0.5, 0.39, 0.27); II: 1.65 (0.6, 0.21, 0.3, 0.33, 0.21); III 1.98 (0.6, 0.26, 0.34, 0.42, 0.36); IV: 2.07 (0.7, 0.36, 0.52, 0.3, 0.19). Epigynum and vulva: septum separating epigynal septum and the two copulatory openings, narrow epigynal septum, anterior epigynal border without a tooth, vulva with a thin translucent septum (Figs. 3F–I).

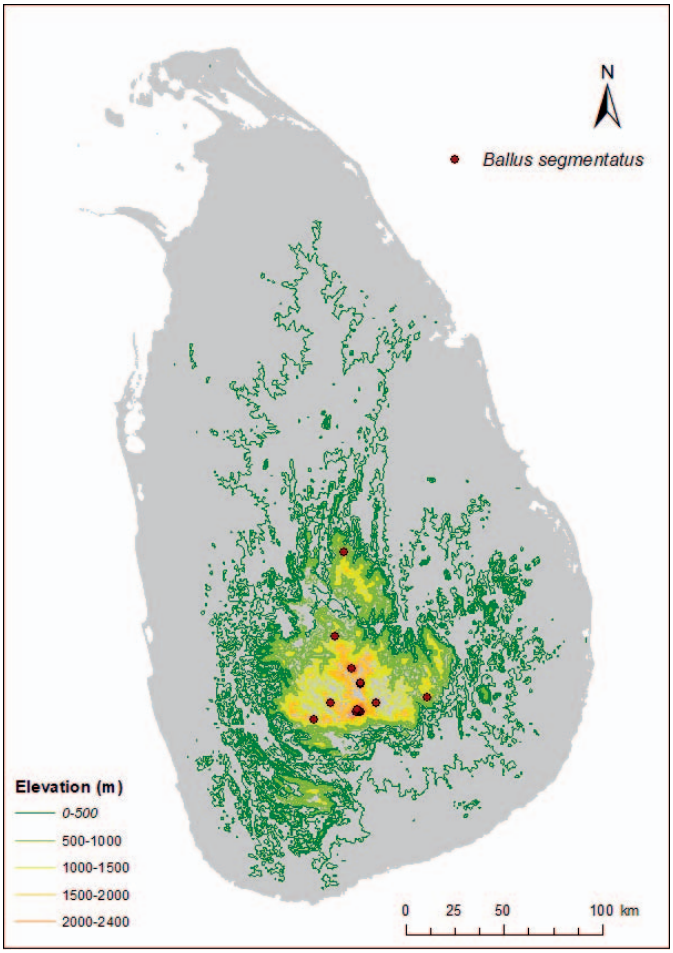

Figure 4.—Collection localities in Sri Lanka of specimens of *Ballus segmentatus* that were used in the molecular species delimitation analyses. Map by Iresha Wijerathne.

Habitat and distribution.—The spiders were collected by beating foliage up to a height of two meters, with a single specimen also collection from leaf litter. The two disjunct populations occur in sub-montane wet evergreen cloud forests and high-elevation montane cloud forests.

Conservation.—*Ballus segmentatus* is known from relatively few individuals and is restricted to mid and high-altitude cloud forest (900–1800m) in the Central and Uva Provinces of Sri Lanka. Most known localities are within protected areas. Conservation status assessment using the IUCN criteria (IUCN 2012) results in a status of vulnerable 'VU B1′. This assessment is based on an estimated geographic range of $<$ 20,000 km^2, fragmented habitat and a number of locations of no more than 10.

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SUPPLEMENTAL MATERIALS

Figure S1.—Individual results from three repeated runs of multi-locus species delimitation with BPP. The cladogram represents the topology of the guide tree that was inferred with IQ-TREE based on the concatenated matrix of all three genes. Branch lengths are meaningless. Three colour-coded squares at nodes illustrate posterior supports of three repeated analyses. Available online at https://doi.org/10.1636/JoA-S-21-040.s1

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