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Discovery of the Sri Lankan Relict Ant, *Aneuretus simoni* Emery (Formicidae, Aneuretinae) and the nest density of the species in a selected region of Meethirigala Forest Reserve, Sri Lanka

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ABSTRACT. The sole extant species of Aneuretinae, *Aneuretus simoni* Emery, forms ground nests in various substrates in wet zone and intermediate zone forests in Sri Lanka. Nests of the species were surveyed outside the previously recorded habitats, in Meethirigala Forest Reserve in Gampaha District. The frequency of occurrence, percentage nest abundance and mean nest density in three localities were investigated by laying 40 quadrats of 0.5 m x 0.5 m at two plots in each locality, and counting the number of nests within each quadrat, from March to July in 2014. Nests of *A. simoni* were found in only one locality. Higher frequency of occurrence (4/40 to 8/40) than observed for other species was observed for *A. simoni* in all samples except in June, and the percentage nest abundance ranged from 5.7 to 20.5. The mean nest density of the species was among the highest four on each occasion and was, along with a *Pheidole* species, significantly higher overall than that of 14 other species. The nest density of *A. simoni* ranged from 0.2 - 0.8 m^{-2} from March to July in the forest, and this was higher than that recorded at its other habitats except the Kuluna Kanda Proposed Forest Reserve. The mean nest density of *A. simoni* had no significant correlation with monthly rainfall. *Aneuretus simoni* is locally-dominant at an altitude of 57 m in Meethirigala Forest Reserve, which is the lowest elevation record for the species.

Keywords: Sri Lankan Relict Ant, endemic ants, quadrat sampling for nests, ground ant nests, forest ant fauna

INTRODUCTION

The Sri Lankan Relict Ant, *Aneuretus simoni* Emery, is the single extant representative of the subfamily Aneuretinae and is endemic to Sri Lanka (Wilson *et al.*, 1956; Jayasuriya and Traniello, 1985; Bolton, 1994) This ant forms nests among the leaf litter, in superficial soil layers, in the recesses in boulders and in dead wood pieces in the disturbed forests and Forest Reserves in Sri Lanka (Wilson *et al.*, 1956; Jayasuriya and Traniello, 1985; Dias *et al*., 2013; Dias and Ruchirani, 2014; Dias, 2014). Workers have generalized foraging habits and are omnivorous scavengers feeding on rotten fruit and nectar (Jayasuriya and Traniello, 1985; Dias *et al*., 2013; Dias and Ruchirani, 2014). It is mainly a litter dwelling ant species.

Aneuretus simoni has been assessed as Critically Endangered globally (Social Insects Specialist Group, 1996) and Endangered locally (Dias *et al*., 2012). In previous records, the species has been reported from the Peradeniya area of Kandy District in Central Province (Wilson *et*

al., 1956), Pompekelle Forest Reserve (Wilson *et al*., 1956; Dias 2004, 2014), Adam's Peak Forest Reserve (Wilson *et al*., 1956), Gilimale Forest Reserve (Jayasuriya and Traniello, 1985; Dias and Perera, 2011) and Sinharaja Forest Reserve (Perera *et al*., 2006; Gunawardene *et al*., 2008); Kirikanda Forest (Dias *et al.,* 2011; Dias *et al*. 2013) and Kalugala Proposed Forest Reserve (KPF) (Dias and Ruchirani, 2014) of southwest Sri Lanka, two forests in Moraella and Rambukoluwa in Central Sri Lanka (Karunarathna and Karunaratne, 2013) and Kuluna Kanda Proposed Forest Reserve (KKPFR) and Wilpita "Aranya Kele" (WFR) in southern Sri Lanka (Dias and Ruchirani, 2014) (Figure 1).

Nest densities of *A. simoni* were reported in Gilimale Forest Reserve in 1979 and the distribution of the species was reported to be associated with an annual rainfall of 2000 - 5000 mm and elevation at least 450 m above mean sea level (Jayasuriya and Traniello 1985). Higher nest densities and lower elevations, e.g. 108 - 250 m in Kirikanda Forest (Dias *et al*., 2013), 95 m at Kalugala Proposed Forest Reserve, were subsequently reported elsewhere (Dias and Ruchirani, 2014). The Meethirigala Forest Reserve (Figure 1), a tropical rain forest of 384 ha from 57 m to 109 m in elevation, is situated in Gampaha District in the Western Province of Sri Lanka. A road runs between the two mountainous fragments. The bird species diversity of this forest is high (Ceylon Bird Club, 2015), but little is known on the diversity of invertebrates.

We report here on the discovery of *A. simoni* nests, frequency of nest occurrence, mean nest density and percentage nest abundance of the species and associated ant species (where *A. simoni* survived) that were observed by repeated quadrat sampling in Meethirigala Forest Reserve from March to July in 2014. This area is at a lower elevation range than most other known habitats of the species.

MATERIALS AND METHODS

The present investigation was carried out in the mountainous forest that lies opposite to the Buddhist monastery in the Meethirigala Forest Reserve (Figure 1).

Ant nests at two, 50 m^2 (10 m × 5 m) plots were surveyed at each of three localities approximately 200 m apart (Table 1). Locality A had a moist, sandy floor and a taller canopy, which shaded the area; subcanopy (e.g. *Lansium parasiticum*) and grasses on the forest floor were moderately developed and the ground was usually covered with a dense leaf litter layer. Locality B had poorly developed canopy layers and well-developed undergrowth layer; a dry forest floor of clay soil was covered with a thin layer of leaf litter and small to large stones and gravel were also seen. Locality C had very poor stratification; irregularly scattered tall trees with narrow stems such as *Alstonia macrophylla* Wall. ex G. Don and *Acacia auriculiformis* A. Cunn. were abundant. The clay forest floor had scattered gravels and a dense dry leaf litter layer. A section in Locality C consisted of a replanted forest (personal communication, field forest officer of Meethirigala Forest Reserve).

Preliminary survey for *A. simoni* workers

Honey baiting, breaking of decaying wood pieces and leaf litter sifting were conducted during day time at Locality A, the lowest elevation (57 m) of Meethirigala Forest Reserve, in February 2014. Fifty pieces of gauze, each of 2 × 2 cm, with a drop of honey were placed throughout the area and were collected after an hour. Decaying logs and pieces of wood were broken and checked for *A. simoni* colonies and workers. Leaf litter collected into a sieve was sifted and the ants falling into the white tray were collected. Collected ants were preserved in the glass bottles (7 ml) filled with 70% ethanol with relevant labels and checked for the presence of *A. simoni* workers under a low-power stereo-microscope. Based on the presence of *A. simoni* in the samples the survey of its nests and associated ants was conducted as given below.

Field survey and laboratory methods

Ant nests at two, 50 m^2 (10 m × 5 m) plots of Locality A (57 m elevation), Locality B (84 m) and Locality C (109 m) of the study region were surveyed during 28-29 March, 26-27 April, 28-29 May, 23-24 June and 30-31 July in 2014. Twenty 0.5 m x 0.5 m quadrats were laid within each plot, at least 1 m apart from each other, by fixing four wooden pegs in the ground and connecting them

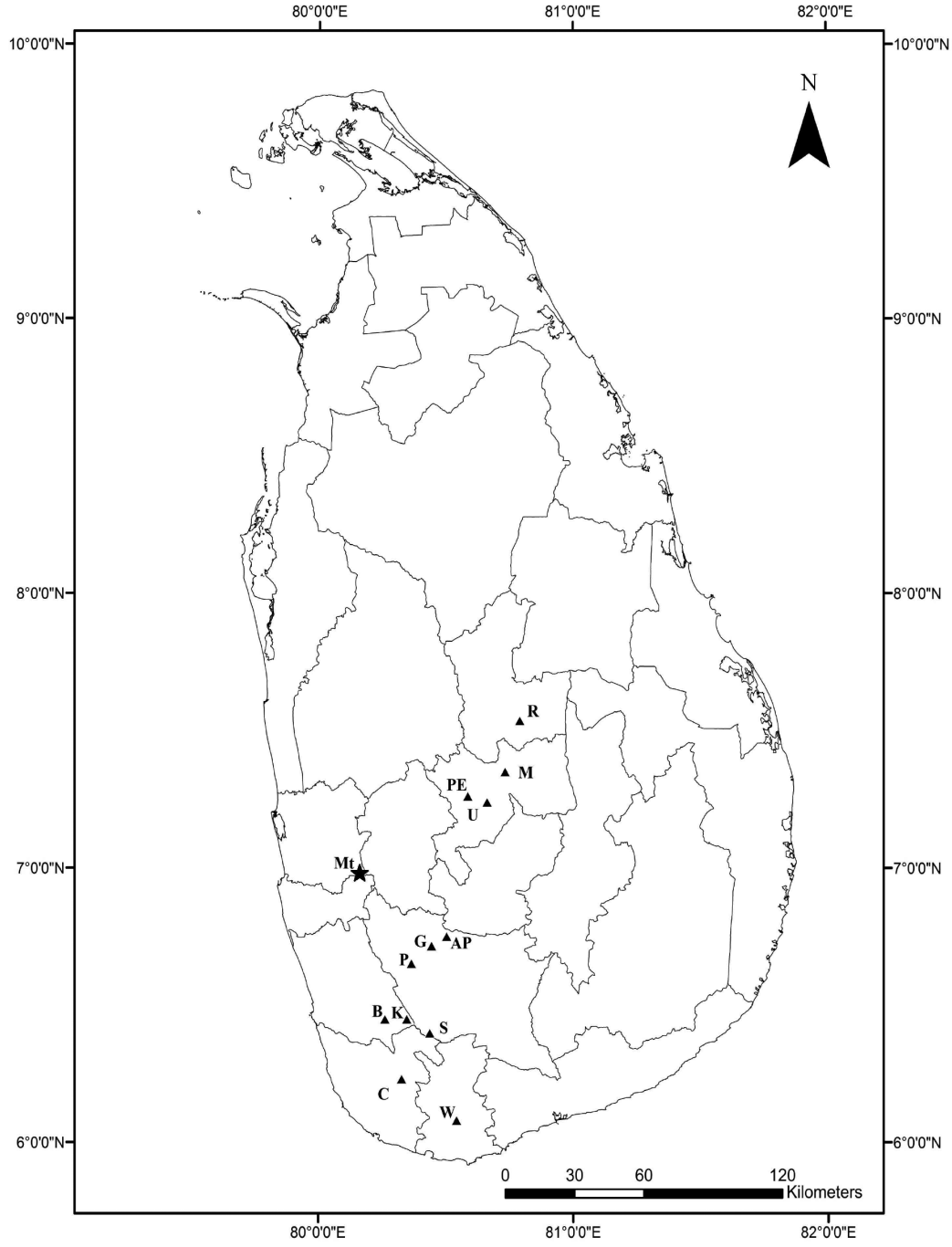

Fig. 1. Map of Sri Lanka showing the previous habitats (marked by triangles) of *A. simoni* and the Meethirigala Forest Reserve (Mt, marked by an asterisk) (modified from Dias and Ruchirani, 2014).
AP - Adam's Peak, B - Kalugala Proposed Forest Reserve, C - Kuluna Kanda Proposed Forest Reserve, G - Gillimale Forest Reserve, K – Kiri Kanda Forest M - Moragahakanda Forest, Mt - Meethirigala Forest Reserve, P - "Pompekelle", PE - Peradeniya, R - Rambukoluwa, S - Sinharaja Forest Reserve, U - Udawaththa Kele, W - Wilpita "Aranya Kele"

Table 1. Altitude and the GPS coordinates of the sampling plot of each locality.

	Collection localites					
	A1	**A2**	**B1**	**B2**	**C1**	**C2**
GPS coordinates	06° 59.769′ N	06° 59.797′ N	06° 59.679′ N	06° 59.686′ N	06° 59.566′ N	06° 59.582′ N
	80° 10.638′ E	80° 10.412′ E	80 10.386′ E	80° 10.392′ E	80° 10.491′ E	80° 10.516 ′ E
Altitude	57 m	57 m	84 m	84 m	109 m	109 m

with a cord. Nests of ants were searched by carefully removing leaf litter and breaking twigs and decaying wood pieces within each quadrat. Ant nests with a single entrance or multiple entrances were encountered depending on the species. Nests formed among leaf litter had many workers with the brood whereas those in superficial soil layer had single hole or multiple entrances close to each other and were considered as one nest. Group of workers and the brood seen in a piece of a single decaying twig was counted as one nest irrespective of the number of holes observed in the twig. The number of nests observed within each quadrat was recorded and three workers from each nest were preserved in glass bottles filled with 70% ethanol. Worker ants in each bottle were identified to the furthest taxonomic level using a low-power stereo-microscope with reference to Bingham (1903), Bolton (1994), and scientific names are presented here according to Bolton (2014). Antennae, mandibles, maxillary palps and legs of workers were dissected out where necessary and observed under a high-power microscope with a drop of glycerol. All voucher specimens are kept in the repository of the first author at the Department of Zoology and Environmental Management, University of Kelaniya, Sri Lanka.

Measurement of environmental parameters

Environmental parameters were measured at three representative places in each plot within three localities, and mean values were calculated. Air and soil temperature was measured using a digital thermometer. The depth of leaf litter was measured using a ruler. Three soil samples from each plot were collected into polythene bags; a known weight of soil from each sample was dried in an oven at 105°C until a steady dry weight was observed, and the soil moisture content was calculated according to Brower *et al*. (1990). Soil organic matter content was determined according to Sutherland (2006). Monthly rainfall data for the nearest available meteorological station at Avissawella from March to July were obtained from the Department of Meteorology in Colombo.

Estimation of nest density, frequency of nest occurrence and association between *A. simoni* nest density and each environmental parameter

The nest density (= number of nests of the species in one locality/ sum of the quadrat areas at the locality (10 m^2)), frequency of nest occurrence (= number of quadrats with nests of the focal species/total number of quadrats laid) and the percentage nest abundance (= number of nests of the focal species/total number of nests of all species) of each species were calculated. Rank-abundance diagrams were drawn for the mean nest density of each ant species observed on the five occasions from March to July following Dias *et al*. (2013). One Way Analysis of Variance followed by Tukey's test (Minitab 14.0) was conducted to test significant differences among the nest density values observed on the five occasions. Any association between the nest density of *A. simoni* and soil temperature, soil moisture content, soil organic matter content and monthly rainfall was tested using Pearson's correlation analysis.

RESULTS

Preliminary observations, frequency of nest occurrence and percentage nest abundance

Workers of *A. simoni* were found in several samples collected by breaking decaying wood pieces and sifting the leaf litter during the preliminary survey. During the main survey from March to July, *A. simoni* workers or nests were encoun-

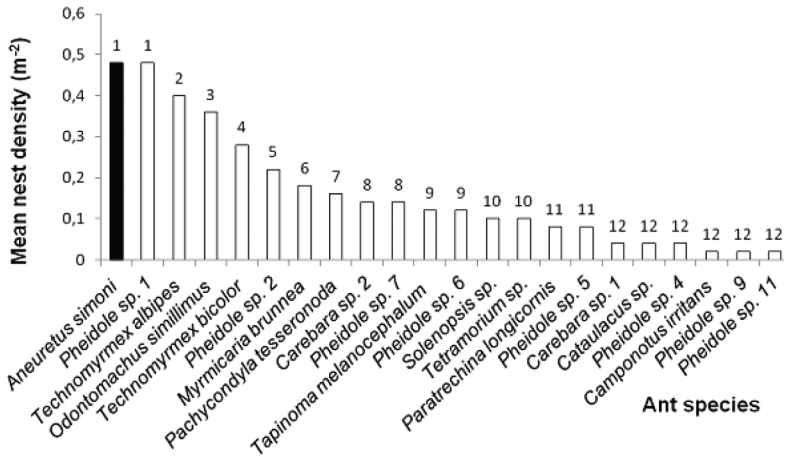

Fig. 2. Ranked overall mean nest density of ant species observed at Locality A for March to July period

tered at Locality A only. Frequency of nest occurrence (FNO) and percentage nest abundance (NA %) of ant fauna at Locality A is presented in Table 2. Higher FNO values for *A. simoni* nests than those observed for the nests of other ant species were noticeable in March and July. The highest frequency of nest occurrence was observed for *Technomyrmex albipes* in April and *Pheidole* sp. 1 in May and June. The highest (8/ 40) and the lowest (2/ 40) frequency of nest occurrence values for the *A. simoni* nests were observed in July and June, respectively.

The NA% of *A. simoni* ranged from 5.7 to 20.5 during the study period (Table 2).

Mean nest density and the rank of *A. simoni*

The mean nest density (MND) observed from March to July at Locality A is shown in Table 2. The MND of *A. simoni* was the highest, and highest-ranked, in March and July and varied from second- to fourth-highest in April, May and June. *Myrmicaria brunnea* and *Odontomachus simillimus* had the second-highest for MND in March and July, respectively. In April, MND of *T. albipes* was highest while *Carebara* sp. had similar MND to that of *A. simoni*. The MND of *Pheidole* sp. 1 and *Pheidole* sp. 2 were first- and second-ranked in May whereas that of *Pheidole* sp. 1, *T. bicolor* and *O. simillimus* had the first, second and third ranks in June. Higher ranks for the MND of other species were observed from March to July. Significant differences were not observed between the MND values of *A. simoni* observed on the five occasions (ANOVA, $p > 0.05$).

Twenty two ant species of 14 genera in 5 subfamilies, Aneuretinae, Dolichoderinae, Formicinae, Myrmicinae and Ponerinae, were observed at Locality A in Meethirigala Forest Reserve from March to July 2014. The overall MND of *A. simoni* and *Pheidole* sp. 1 at Locality A were joint-highest and significantly higher than that of 20 other species (Figure 2). Significant difference was not evident (ANOVA; $p > 0.05$) between the MND of *A. simoni* and that of M. *brunnea*, *O. simillimus*, *Pheidole* sp. 1 and 2, *T. albipes* and *T. bicolor*.

Table 3 shows that the environmental conditions existing at Locality A slightly fluctuated from March to July. Higher soil moisture (%), lower leaf litter and higher rainfall were observed in June. Significant association was not evident (Pearson's Correlation analysis; $p > 0.05$) between the MND of *A. simoni* and each parameter. Among the *A. simoni* nests, eight were observed among leaf litter, seven were observed in the superficial soil layer and five were located in decaying stem pieces of the bamboo, *Fargesia* sp. commonly seen in the area.

Table 2. Ant species that occupied the nests observed at Locality A from March-July at Meethirigala Forest Reserve MND = Mean nest density (m^{-2}),FNO = Frequency of nest occurrence, NA% = Percentage nest abundance

	Ant species	March			April			May			June			July		
		FNO	NA%	MND	FNO	NA%	MND	FNO	NA%	MND	FNO	NA%	MND	FNO	NA%	MND
1	*Aneuretus simoni*	5/40	19.2	0.5	4/40	12.5	0.4	5/40	10.2	0.5	2/40	5.7	0.2	8/40	20.5	0.8
2	*Tapinoma melanocephalum*	1/40	3.8	0.1	2/40	6.3	0.2	–	–	–	–	–	–	3/40	7.7	0.3
3	*Technomyrmex albipes*	4/40	15.4	0.4	9/40	28.1	0.9	2/40	4.1	0.2	2/40	5.7	0.2	3/40	7.7	0.3
4	*Technomyrmex bicolor*	1/40	3.8	0.1	2/40	6.3	0.2	2/40	4.1	0.2	7/40	20.0	0.7	2/40	5.1	0.2
5	*Camponotus irritans*	–	–	–	1/40	3.1	0.1	–	–	–	–	–	–	–	–	–
6	*Paratrechina longicornis*	–	–	–	2/40	6.3	0.2	–	–	–	2/40	5.7	0.2	–	–	–
7	*Carebara* sp. 1	–	–	–	–	–	–	2/40	4.1	0.2	–	–	–	–	–	–
8	*Carebara* sp. 2	-	-	-	4/40	12.5	0.4	3/40	6.1	0.3	–	–	–	–	–	–
9	*Cataulacus* sp.	–	–	–	1/40	3.1	0.1	–	–	–	1/40	2.9	0.1	–	–	–
10	*Myrmicaria brunnea*	4/40	15.4	0.4	–	–	–	–	–	–	2/40	5.7	0.2	3/40	7.7	0.3
11	*Pheidole* sp. 1	–	–	–	3/40	9.4	0.3	9/40	18.4	0.9	8/40	22.9	0.8	4/40	10.3	0.4
12	*Pheidole* sp. 2	–	–	–	1/40	3.1	0.1	7/40	14.3	0.7	1/40	2.9	0.1	2/40	5.1	0.2
13	*Pheidole* sp. 4	–	–	–	–	–	–	1/40	2.0	0.1	1/40	2.9	0.1	–	–	–
14	*Pheidole* sp. 5	–	–	–	2/40	6.3	0.2	–	–	–	–	–	–	2/40	5.1	0.2
15	*Pheidole* sp. 6	3/40	11.5	0.3	–	–	–	1/40	2.0	0.1	1/40	2.9	0.1	1/40	2.6	0.1
16	*Pheidole* sp. 7	–	–	–	–	–	–	4/40	8.2	0.4	–	–	–	3/40	7.7	0.3
17	*Pheidole* sp. 9	1/40	3.8	0.1	–	–	–	–	–	–	–	–	–	–	–	–
18	*Pheidole* sp. 11	–	–	–	–	–	–	1/40	2.0	0.1	–	–	–	–	–	–
19	*Solenopsis* sp.	–	–	–	–	–	–	4/40	8.2	0.4	1/40	2.9	0.1	–	–	–
20	*Tetramorium* sp.	–	–	–	1/40	3.1	0.1	4/40	8.2	0.4	–	–	–	–	–	–
21	*Odontomachus simillimus*	4/40	15.4	0.4	–	–	–	4/40	8.2	0.4	5/40	14.3	0.5	5/40	12.8	0.5
22	*Pachycondyla tesseronoda*	3/40	11.5	0.3	–	–	–	–	–	–	2/40	5.7	0.2	3/40	7.7	0.3

Table 3. Soil temperature (soil T ºC), air temperature (Air T ºC), soil moisture content %, soil organic matter content %, and litter depth (cm) at Locality A in Meethirigala Forest Reserve and monthly rainfall (data from Meteorology Department, Avissawella station) from March to July 2014. *Heavy rain had washed away leaf litter.

2014	Soil T ºC	Air T ºC	Soil moisture%	Soil organic matter%	Litter depth cm	Rainfall mm
28-29 March	25.3±0.0	28.0±0.0	13.3±0.69	5.2±0.07	6.0±0.87	178.9
26-27 April	26.3±0.06	28.0±0.0	15.8±0.43	5.3±0.25	6.0±0.8	284.5
28-29 May	26.9±0.03	27.0±0.0	16.3±0.46	5.6±0.16	6.0±0.43	140.5
23-24 June	26.4±0.03	28.0±0.0	18.3±0.54	5.1±0.29	0.0*	538.2
30-31 July	26.1±0.49	28±0.0	17.5±0.48	4.33±0.35	4.0±0.24	194.6

DISCUSSION

The presence of *A. simoni* nests in Meethirigala Forest Reserve was reported for the first time and this is the first record from Gampaha District. The nest density of *A. simoni* recorded at Locality A (57 m elevation) of Meethirigala Forest Reserve was lower than that recorded from Kuluna Kanda Proposed Forest Reserve (Dias and Ruchirani, 2014) but higher (except in June) than those observed at Gilimale Forest (Jayasuriya and Traniello, 1985), Kirikanda Forest (Dias *et al*., 2013), Kalugala Proposed Forest Reserve and Wilpita "Aranya Kele" (Dias and Ruchirani, 2014) except in June. The rank of *A. simoni* nest density at Locality A was the highest among those observed for the ant species on all occasions except in June showing that it was a major component of the ant community in Locality A. It was found only at Locality A and therefore, may be a minor component at the whole study region in the forest. Absence of ideal substrates for nesting due to the exposure to direct sunlight at Locality B and very low levels of soil moisture with dry substrates at Locality C, perhaps, lead to the devoid of *A. simoni* nests at the two localities. Variation of the rank of the nest density of *A. simoni* observed with the occasion was, most probably, due to rainfall (exact figure for each locality was not available) followed by leaf litter depth at the localities (no leaf litter in June shown in Table 3).

Earlier, *A. simoni* has been reported from elevations above 450 m (Jayasuriya and Traniello, 1985) but it has been also recorded at lower elevations as low as 95 m (in Kalugala Proposed Forest Reserve; Dias *et al.*, 2014), recently. Occurrence of the nests of *A. simoni* at 57 m elevation was recorded for the first time and the range of elevation recently recorded for the species can be considered 57 m (current finding) to 250 m elevation ("Kirikanda" Forest) in the wet zone of Sri Lanka.

Meethirigala Forest Reserve receives a mean annual rainfall of about 2000 - 3000 mm (personal communication, Department of Meteorology, Colombo, Sri Lanka, 31 July, 2014) which is comparable with the range of annual rainfall of the previously recorded habitats, 2000 – 5000 mm in Gilimale Forest Reserve (Jayasuriya and Traniello, 1985), 2000-3000 mm in Kirikanda forest (Dias *et al*., 2013) but lower than the annual rainfall, 4000-5000 mm in Kalugala proposed Forest Reserve (Dias *et al*., 2014). Soil temperature between 25°C and 27°C, air temperature between 27°C and 29°C and litter depth between 2 cm and 6 cm observed at the time of detection of *A. simoni* at Meethirigala Forest Reserve were comparable with those recorded in Gilimale Forest Reserve (Dias and Perera, 2011), Sinharaja Forest Reserve (Perera *et al.*, 2006) and Kirikanda Forest (Dias *et al.*, 2013) but a lower range of soil moisture content, 13% – 18%, was observed at Meethirigala Forest Reserve.

Similar types of microhabitats to that reported in other habitats of *A. simoni* such as hollow cavities of decaying fallen twigs, leaf litter, bark of rotting logs and superficial layer of soil (Wilson *et al*., 1956; Jayasuriya and Traniello, 1985; Dias *et al*., 2001; Dias, 2004; Perera and Dias, 2004) and flat rock surfaces (Dias and Ruchirani, 2013) were observed at the current locality. Decaying stems of a *Fargesia* sp. were

identified as nesting substrate for the first time during the survey. There is an urgent need for the re-assessment of the local and global status of *A. simoni*, after conducting surveys in the forests in two other remaining wet-zone Districts, Colombo and Kegalle, in Sri Lanka, in the future.

CONCLUSION

Aneuretus simoni was a dominant resident species with a nest density ranging from 0.2 to 0.8 m^{-2} at Locality A, 57 m elevation, of Meethirigala Forest Reserve in the Western Province of Sri Lanka in 2014.

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