PREPRINT

Author-formatted, not peer-reviewed document posted on 11/12/2023

DOI: https://doi.org/10.3897/arphapreprints.e117085

Integrative taxonomy reveals first record of invasive *Loxosceles rufescens* (Dufour, 1820) (Araneae, Sicariidae) in the Philippines

Aimee Lynn Barrion-Dupo, Ireneo, Jr. Lit, Camille Faith Duran, Ma. Francia Kyla Cammayo, Marnelli Alviola, Sheila Mae Mercado, Cecille Ann Osio, Orlando Eusebio, Cristian Lucañas, Alberto Barrion

ARPHA Preprints *Author-formatted, not peer-reviewed document posted on 11/12/2023*. DOI: https://doi.org/10.3897/arphapreprints.e117085

Integrative taxonomy reveals first record of invasive *Loxosceles rufescens* (Dufour, 1820) (Araneae, Sicariidae) in the Philippines

Aimee Lynn A. Barrion-Dupo[‡,§], Ireneo, Jr. L. Lit[‡,§], Camille Faith D. Duran[‡], Ma. Francia Kyla M. Cammayo[‡], Marnelli S. Alviola[‡,§], Sheila Mae Q. Mercado[§], Cecille Ann L. Osio[§], Orlando L. Eusebio[‡], Cristian C. Lucañas[‡], Alberto T. Barrion[|]

‡ Entomology Section, Museum of Natural History, University of the Philippines Los Baños (UPLB), Los Baños, Laguna, Philippines
§ Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños (UPLB), Los Baños, Laguna, Philippines
| Department of Biology, College of Science, De La Salle University, Taft, Manila, Philippines

Corresponding author: Aimee Lynn A. Barrion-Dupo (abdupo@up.edu.ph)

Abstract

Background

The spider family Sicariidae Keyserling, 1880, and the synanthropic Mediterranean recluse spider, *Loxosceles rufescens* (Dufour, 1820), is reported in the Philippines for the first time based on morphological and molecular data. The introduced spider was observed in a small cave (Kamantigue Cave) in Lobo, Batangas Province. Considering the medical importance of this spider, the proximity of its habitat to human habitation and tourist sites poses a potential public health concern.

New information

This study reports on the first record of the family Sicariidae in the Philippines and the third recorded occurrence of *L. rufescens* in Southeast Asia.

Keywords

caves, *Loxosceles rufescens*, loxoscelism, Mediterranean recluse

Introduction

Spiders are among the most widely distributed organisms in the world, with more than 50,000 recorded valid species belonging to 132 families (World Spider Catalog 2023). In the Philippines, the documented spider fauna is usually limited to agricultural areas (Barrion and Litsinger 1995). Studies of species in cave and karst environments are relatively recent, the most notable being those of Barrion-Dupo et al. (2014), Rasalan et al. (2015), Maandig et al. (2017), and Rasalan and Barrion-Dupo (2019). Fieldwork and other research activities in several areas within Southern Tagalog (CALABARZON) Region in Luzon are steadily accumulating material toward more comprehensive treatment of spiders and allies in the area. Here, we report the first field observations of a notable population of *Loxosceles rufescens* Dufour, 1820 (Dufour 1820) found in a cave in Lobo, Batangas, Philippines.

Loxosceles Heineken & Lowe, in Lowe (1832), also known as recluse or violin spiders, due to the distinctive violin-shaped mark on the cephalothorax, are characterized by their slanted legs when at rest and by having six eyes arranged in pairs (Vetter and Visscher 1998, Lotz 2017, Trivedi and Dal 2019). The genus currently consists of 143 species (World Spider Catalog 2023). These spiders are known to inflict dangerous bites, known as loxoscelism which can cause necrotic skin lesions, dermonecrosis, skin ulceration, and sometimes, death (Nentwig et al. 2017, Zamani et al. 2020).

Loxosceles rufescens or the Mediterranean recluse originated from North Africa and became naturalized in the circum-Mediterranean region (Gertsch and Ennik 1983, Duncan et al. 2010) but is now considered cosmopolitan as it has already spread across various temperate and tropical countries and regions like the USA, Asia, Australia, Atlantic region, Madagascar, and the Hawaiian Islands (Gertsch and Ennik 1983, Vetter 2008, Planas et al. 2014). In Southeast Asia, it was recently reported to occur in caves in Thailand and in Laos (Chomphuphuang et al. 2016, Jäger 2007).

Materials and methods

Specimens were collected from Kamantigue Cave, Barangay Biga, Lobo, Batangas on 10-13 September 2022 and 9-13 November 2022 under the Department of Environment and Natural Resources Wildlife Gratuitous Permit (DENR WGP): R4A-WGP-2021-BAT-006. Photographs of spiders in their natural habitat were taken using a Nikon D3100 with a macro lens. All specimens were deposited in the Entomology Section, Museum of Natural History, University of the Philippines Los Baños (UPLB-MNH).

Kamantigue Cave (Fig. 1) is located a few meters from the seashore and a few meters adjacent to residential houses. Based on DENR Memorandum Circular No. 2021-193, the cave is classified as Class I due to its extremely hazardous conditions.

The cave has three low and narrow entrances and multiple narrow chambers with abundant guano deposits. The temperature inside the cave is notably high and air flow is very limited. Portions of the cave have collapsed and eroded rocks blocked some of its chambers.

Collected specimens were submerged in 80% ethanol, examined, and measured under a Nikon SMZ 800 stereomicroscope, fitted with an ocular micrometer. Body measurements followed the standard procedure adopted by Barrion and Litsinger (1995). All measurements are in mm. Male and female copulatory organs were examined prior to dissection. The right pedipalp of the male was cut and photographed apically, prolaterally, and retrolaterally using an Olympus Z61 stereomicroscope mounted with a digital camera. The female epigynum was cut from the abdomen and cleared in 10% KOH for 15-20 mins. It was then washed with distilled water three times and transferred to glycerine.

Confirmation of species identification was also performed via molecular analysis of the mitochondrial cytochrome oxidase subunit I (*COI*) gene. Genomic DNA was extracted from the whole specimen using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), following the manufacturer's protocol. The *COI* gene was amplified using the forward (5′-GGAGGATTTGGAAATTGATTAGTTCC-3′) and reverse (5′-CCCGGTAAAATTAAAATATAAACTTC-3′) primers designed by Simon et al. (1994). The 25-μL PCR reaction tubes, each containing the 1× GoTaq® Colorless Master Mix (Promega), 0.1 mM forward and reverse primer, ≤250 ng DNA template, and nuclease-free water, were placed in the Bioer GeneExplorer™ Thermal Cycler (Alpha Laboratories, Eastleigh, UK). The cycling profile was as follows: initial denaturation at 95°C for 2 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 95°C for 30 s, and extension at 46°C for 1 min; final extension at 72°C for 2 min; and holding at 4°C for 10 min. The PCR products were sent to Macrogen (Seoul, South Korea) for standard DNA sequencing.

The raw sequences were preprocessed by trimming the ends to remove the low-quality bases (<10 quality scores) and editing the ambiguous bases using Chromas 2.6.6 (http://technelysium.com.au/wp/chromas/). The nucleotide basic local alignment search tool (BLASTn, Altschul et al. 1990) was used to determine sequence similarity with the publicly available sequences in the National Center for Biotechnology Information (NCBI) database (Table 1). Sequence alignment was performed using MUSCLE (Edgar 2004), followed by phylogenetic analysis using MEGA 11 software (Tamura et al. 2021). A consensus tree was generated using the unweighted pair group method with arithmetic mean (UPGMA) and 1,000 bootstrap replications.

Taxon treatment

Loxosceles rufescens (Dufour, 1820)

Nomenclature

Scytodes rufescens Dufour, 1820 - Dufour (1820): 203; For full synonymy see World Spider Catalog.

Materials

a. country: Philippines; stateProvince: Batangas; municipality: Lobo; locality: Brgy. Biga, Kamantigue Cave; verbatimLocality: PHILIPPINES: Luzon, Batangas, Lobo, Biga: Kamantigue Cave; eventDate: 2022-09-11; year: 2022; month: 09; day: 11; habitat: Camantigue Cave; individualCount: 5; sex: 2 males, 3 females; lifeStage: adult; preparations: in EtOH; catalogNumber: ARA-00526, 527; 528-530; identificationID: Loxosceles rufescens; identifiedBy: NICER P3; type: PhysicalObject; institutionID: UPLB MNH; institutionCode: UPLBMNH; occurrenceID: 617D84B3-4CE3-58F4-980F-0AD6ABA92187

b. country: Philippines; stateProvince: Batangas; municipality: Lobo; locality: Brgy. Biga, Kamantigue Cave; verbatimLocality: PHILIPPINES: Luzon, Batangas, Lobo, Biga: Kamantigue Cave; eventDate: 2022-09-11; year: 2022; month: 11; day: 19; habitat: Camantigue Cave; individualCount: 4; sex: 1 male, 2 females, 1 juvenile; lifeStage: 3 adults, 1 juvenile; preparations: in EtOH; catalogNumber: ARA-00531, 532, 533, 534; identificationID: Loxosceles rufescens; identifiedBy: NICER P3; type: PhysicalObject; institutionID: UPLB MNH; institutionCode: UPLBMNH; occurrenceID: 31CBAEC6-CBA0-5A85-B4C4-BC53671C4089

Description

Male (Fig. 2A). Total length 8.05 $\pm$ 0.92. Carapace: length 4.30 $\pm$ 0.14, width 3.40 $\pm$ 0.57, height 1.75 $\pm$ 0.21. Abdomen: length 3.75 $\pm$ 1.06, width 2.18 $\pm$ 0.46, height 2.08 $\pm$ 0.18. Pedipalp length 3.75; femur 1.40 long; patella 0.45; tibia 1.00 long, 0.60 wide; cymbium 0.50 long, 0.40 wide; bulb 0.40 long. 0.45 wide and embolus 0.50 long.; palpal tibia length /width ratio 1.67. Embolus (1.19 + 0.01) as long as width of globular bulb (1.16 + 0.01).

Carapace pale orange brown marked with dorsal dark orange brown violin-shaped marking. Eyes six in three dyads in a recurved transverse row. Sternum pale yellowish to cream. Chelicerae, labium, and maxillae reddish-brown. Legs orange brown. Abdomen ground color cream brown to grayish brown, with short, gray setae. Leg formula 2-1-4-3 (Table 2). Male palp. Cymbium noticeably shorter than tibia length (0.40:1.00), slightly longer than palp bulb. Embolus possessing a thin cylindrical shaft toward apex (Fig 2B-2D). Female. Habitus as in (Fig. 2E) Total length 8.10 $\pm$ 1.63. Carapace: length 3.38 $\pm$ 0.62, width 2.83 $\pm$ 0.42, height 1.86 $\pm$ 0.15. Abdomen: length 4.73 $\pm$ 1.17, width 2.85 $\pm$ 0.66, height 3.05 $\pm$ 0.90. Coloration and eye arrangement same as male. Leg formula as in male (Table 2). Spermatheca (Fig. 2F) short and rounded distally, its anterior end rounded and directed towards each other (converging) and basal area relatively wide.

Distribution

Southern Europe, northern Africa to Iran, Afghanistan. Introduced to the USA, Mexico, Peru, Macronesia, South Africa, India, China, Japan, Korea, Laos, Thailand, Australia, Hawaii, Philippines (new record).

Notes

This represents a new record of the family Sicariidae Keyserling, 1880, and species in the Philippines. It can be distinguished from other spider families in the Philippines by the six eyes arranged in three dyads in a recurved row, relatively flat carapace, rounded abdomen and tarsal claws two (compared to Scytodidae: humped carapace, tarsal claws 2-3; and 6-eyed Pholcidae: eyes arranged in two distinct triads, abdomen usually elongate and narrow, tarsal claws 2-3).The Philippine specimens exhibit the typical spermatheca and male palp features of the *rufescens*-species group (Binford et al. 2008, Fukushima et al. 2017). Brignoli (1969) and Zamani et al. (2020) noted several variations on the epigyne of *L. rufescens* in Mediterranean, and Iran, Afghanistan and Turkmenistan but refrained from describing them as distinct species without additional specimens.

The spermatheca of Philippine specimens are short and rounded distally with reduced or absent spermathecal bilobation, similar to those from Balkan Peninsula (Naumova and Deltshev 2021), Mexico (Valdez-Mondragón et al. 2018), and from Hormozgan in Iran (Zamani et al. 2020), but slightly differs in the size of the inner receptacle lobe. It also closely resembles the Australian specimen recovered in the Iberian Clade by Duncan et al. (2010).

Similarly, the male palp of Philippine specimens conforms with the report of Lotz (2017) of *L. rufescens*: (a) the short cymbium; (b) cymbium slightly longer than palp bulb; (c) ratio of palp tibia length/height is 1.67; (d) palp cymbium noticeably shorter than the tibia [0.40:1.00]; and (d) embolus possessing a thin cylindrical shaft towards the apex (Fig. 2B-D).

Overall, the examined morphological characters of the Philippine species conform with the present description of *L. rufescens* as presented in Lotz (2017), Naumova and Deltshev (2021), Valdez-Mondragón et al. (2018), and Zamani et al. (2020).

Furthermore, the results of the molecular analysis corroborate those obtained using classical morphological techniques. The BLASTn results of the *COI* sequences generated from four Philippine spider specimens (412–433 bp long) reveals significantly high similarity (percent identity = 98–100%) with those of *L. rufescens*.

Meanwhile, the pairwise distance between the Philippine samples and those from Mediterranean samples are all less than 0.1 (Table 3).

Phylogenetic analysis shows three major clusters consisting of *L. rufescens* specimens (100% bootstrap support), Canarian species (61% bootstrap), and *L. persica* (81% bootstrap) (Fig. 3). In the *L. rufescens* cluster, the specimens from Kamantigue Cave, Lobo, Batangas (PH1–PH4) are grouped together with specimens from Portugal (PT), Spain (SS), and Gran Canaria (GC). This group is the sister clade of *L. rufescens* specimens from Turkey (KT), Italy (SI), and Spain (CS, VS). The four Canarian species cluster with *L. foutadjalloni* but have 30% bootstrap support. The three *L. persica* specimens from Iran are predictably grouped with its sister species, *L. mrazig*.

Discussion

The genus *Loxosceles* Heineken & Lowe, in Lowe (1832), is known to occur in the temperate areas of South Africa, in the tropics, in the Mediterranean region and Southern Europe. In America, it ranges from the temperate and tropical regions of North and South America (Gertsch and Ennik 1983). *Loxosceles rufescens* originated from North Africa, probably Morocco, and transported to its native area within the Mediterranean basin through human transport and through its own means (Duncan et al. 2010, Planas et al. 2014, Nentwig et al. 2017). The unintentional introduction of this spider through human-mediated transport also brought them to the United States (Gertsch and Ennik 1983, Massa et al. 2018). Currently, the species is now spread through the islands of the Atlantic, Madagascar, Hawaii, in the areas of Australia, Mexico, Iran, Afghanistan, Peru, Macaronesia, and South Africa (Chomphuphuang et al. 2016, Gertsch and Ennik 1983, World Spider Catalog 2023). In Asia, it has been reported to occur in Iran (Zamani et al. 2020), India (Trivedi and Dal 2019, Sahoo et al. 2022), China (Chen and Gao 1990), Russia (Dunin 1992), Taiwan (Song et al. 1999), Japan (Ono 2009), South Korea (Namkung 2003). In Southeast Asia, it is reported to be observed in caves in Laos (Jäger 2007) and in Thailand (Chomphuphuang et al. 2016). Zamani et al. (2020) noted the possibility that some of these species (i.e., those from China) might belong to a different species. With this distribution, the species is now considered cosmopolitan.

Loxosceles rufescens are usually found in caves, under rocks, and leaf litter in its Mediterranean distribution (Simon 1914). Reports outside its region of origin, found them living in favor of urban areas such as residential houses, apartments, university buildings, basements, tunnels, and other cave-like human-made structures. Their microhabitats are corner walls, storage boxes, electric meter box, crevices of unused cupboards (Gertsch and Ennik 1983, Trivedi and Dal 2019). They are also found under logs, leaves of wild shrubs, leaf litter, and rocks near urban environments (Sahoo et al. 2022).

In more recent studies outside the Mediterranean region, *L. rufescens* is reported to be also found in caves. In Laos, they were documented to occur in two caves, The Pak Ou (Tham Phun) and Tham Sing Mang Caves, which are both dry caves (Jäger 2007). The species was also found in Tum-Wangpra Cave in Thailand (Chomphuphuang et al. 2016).

It is said to be the first report of *L. rufescens* in natural habitat outside of its native Mediterranean region (Chomphuphuang et al. 2016). Interestingly, these caves are all reported to be dry and have either high temperature or are said to have warmer ground surface.

The abiotic conditions (e.g., precipitation and temperature) used by Taucare-Ríos et al. (2018) to create the global model of distribution for *L. rufescens* predicted that the probable sink population may be present in North Luzon, particularly, the Ilocos Sur region. Our current observation deviates from this model, as *L. rufescens* was collected in Southern Luzon, specifically in the Kamantigue Cave of Barangay Biga, Lobo, Batangas (Fig. 4). The presence of this spider beyond its native range may be an unintentional introduction as chance passengers during travel, trade, and transport.

The spider can be easily spread through human means as they are able to resist long periods of starvation and they are highly synanthropic (undomesticated and lives in close association with humans and benefits from their habitats, surroundings, and activities; Massa et al. 2018). They prefer to hide in wooden objects, cardboard boxes, containers, and other structures that have small crevices, which make them easy to transport along with other goods (Nentwig et al. 2017). The source and means of the introduction of *Loxosceles rufescens* found in the Philippines are still unknown.

Invasiveness in the environment. The cave where *L. rufescens* is found is full of another invasive species, the American cockroach, *Periplaneta americana* (L.), which are known prey for these spiders (Greene et al. 2009), but individuals were observed to feed also on the pholcid, *Smeringopus pallidus* (Blackwall 1858) Fig. 4F), and even other *L. rufescens* (and hence, maybe cannibalistic) that are also present in the cave. The spider is also known to be potentially harmful to humans.

Despite their invasiveness and potential harm, *L. rufescens* does not disperse on their own means easily. All *Loxosceles* species have a low dispersal capacity and do not balloon like other spiders (Vetter 2008, Greene et al. 2009, Massa et al. 2018). They are usually sedentary and prefer to stay in the same location for long periods of time (Massa et al. 2018). In casual conversations with the locals from the residential houses near the cave, they said that there are no sightings of *L. rufescens* in their houses.

Medical importance. The genus *Loxosceles* is among the known dangerous spiders in the world (Sadeghi et al. 2017). All *Loxosceles* species are considered medically important due to their ability to cause skin injury. Their bites can cause local erythema to necrotic skin reactions. In rare cases, it can lead to “loxoscelism”, a disease that causes myalgia, arthralgia, hemolysis, hemoglobinuria, acute renal failure, amputation, and even death (Bajin et al. 2011, Köse et al. 2021).

In most cases, most bites do not result to serious and fatal skin injuries. Most are typically mild and self-healing (Vetter 2008). In severe manifestation of the bite, loxoscelism lesions can grow up to 40 cm in size and can be life threatening especially in children (Vetter 2008). Bites are described as painless and usually occur at night due to the nocturnal nature of

Loxosceles spiders. Typically, these spiders only bite for defensive purposes (Vetter 2008, Nentwig et al. 2017).

Implication in cave classification and management. Kamantigue Cave located at Barangay Biga, Lobo, Batangas is currently classified as Class I due to extremely hazardous conditions such as its bad air condition and presence of rock fall (Department of Environment and Natural Resources 2021). The presence of a viable population of medically important *L. rufescens* is an additional reason to restrict caving activities because therapy for Mediterranean recluse spider envenomation still requires development (Sadeghi et al. 2017).

Acknowledgements

We thank the Philippine Department of Environment and Natural Resources, Region IV-A Office for the grant of Gratuitous Permits (R4A-WGP-2021-BAT-006) which enabled us to collect in the Kamantigue Cave of Lobo, Batangas; the Department of Science and Technology (DOST) for the financial support through the Niche Centers in the Regions for Research and Development (NICER): Center for Assessment of Cave Ecosystems (CAVES) in CALABARZON Project 3 entitled “Terrestrial Arthropod Diversity Assessment of Cave and Karst Areas in CALABARZON”; the UPLB Museum of Natural History for hosting the said project under the UPLB MNH Cave Biodiversity Research Program; the Institute of Biological Sciences, CAS, UPLB, specifically the Environmental Biology Division, Cave Ecology Laboratory, and Genetics and Molecular Biology Division – Molecular Biology and Biotechnology Laboratory (Molecular Genetics) for the use of space, laboratory supplies, and facilities. This paper is also part of the series of outputs in our effort to document the diversity of Philippine terrestrial arthropods under the Philippine Terrestrial Arthropods Biodiversity Survey (PhilTABS). Lastly, we also thank the local government units of Lobo and Barangay Biga for hosting us during our fieldwork.

References

- Altschul S, Gish W, Miller W, Myers E, Lipman D (1990) Basic local alignment search tool. Journal of Molecular Biology 215 (3): 403-410. https://doi.org/10.1016/s0022-2836(05)80360-2
- Bajin MS, Arikan G, Parlak M, Tuncok Y, Yigit N, Durak I, Saatci AO (2011) Necrotic arachnidism of the eyelid due to *Loxosceles rufescens* spider bite. Cutaneous and Ocular Toxicology 30 (4): 302-305. https://doi.org/10.3109/15569527.2011.577494
- Barrion AT, Litsinger JA (1995) Riceland Spiders of South and Southeast Asia. Wallingford, CAB International, 700 pp.
- Barrion-Dupo AL, Barrion AT, Rasalan JB (2014) A new cave-dwelling mygalomorph spider of the genus *Phlogiellus* Pocock, 1897 (Araneae: Theraphosidae: Selenocosmiinae) from Burdeos, Polillo Island, Quezon Province, Philippines. Philippine Journal of Systematic Biology 3: 1-15.

- Binford G, Callahan M, Bodner M, Rynerson M, Núñez PB, Ellison C, Duncan R (2008) Phylogenetic relationships of *Loxosceles* and *Sicarius* spiders are consistent with Western Gondwanan vicariance. Molecular Phylogenetics and Evolution 49 (2): 538-553. https://doi.org/10.1016/j.ympev.2008.08.003
- Blackwall J (1858) Descriptions of six newly discovered species and characters of a new genus of Araneida. Annals and Magazine of Natural History III 1 (6): 426-434. https://doi.org/10.1080/00222935808696953
- Brignoli PM (1969) Note sugli Scytodidae d'Italia e Malta (Araneae). Fragmenta Entomologica 6: 121-166.
- Chen XE, Gao JC (1990) The Sichuan farmland spiders in China. Sichuan Science and Technology Publishing House, Chengdu, 226 pp.
- Chomphuphuang N, Deowanish S, Songsangchote C, Sivayyapram V, Thongprem P, Warrit N (2016) The Mediterranean recluse spider *Loxosceles rufescens* (Dufour, 1820) (Araneae: Sicariidae) established in a natural cave in Thailand. Journal of Arachnology 44 (2): 142-147. https://doi.org/10.1636/r15-61
- Department of Environment and Natural Resources (2021) Memorandum Circular 2021-193. Additional list of Classified Caves for 2020 and Errata on DMC 2014-03 and 2019-09. 1-6.
- Dufour L (1820) Descriptions de cinq arachnides nouvelles. Annales Générales des Sciences Physiques 5: 198-209.
- Duncan R, Rynerson M, Ribera C, Binford G (2010) Diversity of *Loxosceles* spiders in Northwestern Africa and molecular support for cryptic species in the *Loxosceles rufescens* lineage. Molecular Phylogenetics and Evolution 55 (1): 234-248. https://doi.org/10.1016/j.ympev.2009.11.026
- Dunin PM (1992) The spiders family Scytodidae of the USSR fauna. Trudy Zoologicheskogo Instituta Akademii Nauk SSSR, Leningrad 226: 74-82.
- Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32 (5): 1792-1797. https://doi.org/10.1093/nar/gkh340
- Fukushima CS, de Andrade RMG, Bertani R (2017) Two new Brazilian species of *Loxosceles* Heinecken & Lowe, 1832 with remarks on *amazonica* and *rufescens* groups (Araneae, Sicariidae). ZooKeys 667: 67-94. https://doi.org/10.3897/zookeys.667.11369
- Gertsch WJ, Ennik F (1983) The spider genus *Loxosceles* in North America, Central America, and the West Indies (Araneae, Loxoscelidae). Bulletin of the American Museum of Natural History 175: 264-360.
- Greene A, Breisch NL, Boardman T, Pagac BB, Kunickis E, Howes RK, Brown PV (2009) The Mediterranean recluse spider, *Loxosceles rufescens* (Dufour): an abundant but cryptic inhabitant of deep infrastructure in the Washington, D.C. Area (Arachnida: Araneae: Sicariidae). American Entomologist 55 (3): 158-167. https://doi.org/10.1093/ae/55.3.158
- Jäger P (2007) Spiders from Laos with descriptions of new species (Arachnida: Araneae). Acta Arachnologica 56 (1): 29-58. https://doi.org/10.2476/asjaa.56.29
- Köse A, Abacı E, Bozkurt Babus S, Yazıcı A (2021) Skin necrosis, diffuse urticaria, and cellulitis due to presumed *Loxosceles* spider bite. Wilderness & Environmental Medicine 32 (2): 198-203. https://doi.org/10.1016/j.wem.2020.12.005

- Lotz LN (2017) An update on the spider genus *Loxosceles* (Araneae: Sicariidae) in the Afrotropical region, with description of seven new species. Zootaxa 4341 (4): 475-494. https://doi.org/10.11646/zootaxa.4341.4.2
- Lowe RT (1832) Descriptions of two species of Araneidae, natives of Madeira. The Zoological Journal 5: 320-323.
- Maandig RJ, Nunez OM, Barrion-Dupo AL (2017) Species richness and microhabitats of spiders in caves of Agusan del Sur, Philippines. Uttar Pradesh Journal of Zoology 37: 10-21.
- Massa M, Planas E, Ribera C (2018) The Mediterranean as a melting pot: Phylogeography of *Loxosceles rufescens* (Sicariidae) in the Mediterranean Basin. PLOS One 13 (12). https://doi.org/10.1371/journal.pone.0210093
- Namkung J (2003) The Spiders of Korea. 2nd. Kyo-Hak Publishing Co., Seoul, 648 pp.
- Naumova M, Deltshev C (2021) New faunistic and taxonomic notes on the Haplogyne and Cribellate spiders (Araneae: Dictynidae, Dysderidae, Eresidae, Filistatidae, Sicariidae) from three Balkan countries. Acta Zoologica Academiae Scientiarum Hungaricae 67 (1): 63-76. https://doi.org/10.17109/azh.67.1.63.2021
- Nentwig W, Pantini P, Vetter R (2017) Distribution and medical aspects of *Loxosceles rufescens*, one of the most invasive spiders of the world (Araneae: Sicariidae). Toxicon 132: 19-28. https://doi.org/10.1016/j.toxicon.2017.04.007
- Ono H (2009) The spiders of Japan with keys to the families and genera and illustrations of the species. Tokai University Press, Kanagawa, 739 pp.
- Planas E, Ribera C (2014) Uncovering overlooked island diversity: colonization and diversification of the medically important spider genus *Loxosceles* (Arachnida: Sicariidae) on the Canary Islands. Journal of Biogeography 41 (7): 1255-1266. https://doi.org/10.1111/jbi.12321
- Planas E, Saupe EE, Lima-Ribeiro MS, Peterson AT, Ribera C (2014) Ecological niche and phylogeography elucidate complex biogeographic patterns in *Loxosceles rufescens* (Araneae, Sicariidae) in the Mediterranean Basin. BMC Evolutionary Biology 14 (1). https://doi.org/10.1186/s12862-014-0195-y
- Rasalan J, Barrion-Dupo A (2019) Description of a new species of *Masteria* L. Koch, 1873 (Dipluridae: Masteriinae) with a World Catalogue. Journal of Asia-Pacific Biodiversity 12 (2): 160-164. https://doi.org/10.1016/j.japb.2019.01.002
- Rasalan JB, Barrion-Dupo AL, Bicaldo PR, Sotto MP (2015) Spider assemblages of Puting Bato Cave 3-4 and surrounding karst forest environs, with additional notes on the cave-dwelling nature of *Phlogiellus kwebaburdeos*. Museum Publications in Natural History 4: 18-25.
- Ribera C, Planas E (2009) A new species of *Loxosceles* (Araneae, Sicariidae) from Tunisia. ZooKeys 16: 217-225. https://doi.org/10.3897/zookeys.16.232
- Sadeghi S, Dashan M, Malek-Hossein MJ (2017) Mediterranean recluse spider, *Loxosceles rufescens* (Araneae: Sicariidae) from Charkhab Cave, Southern Iran. Journal of Arthropod-Borne Diseases 11 (1): 156-160.
- Sahoo S, Mishra G, Seth JK, Murmu LK, Goud S (2022) New record of the Mediterranean recluse spider *Loxosceles rufescens* (Dufour) from Eastern India. Indian Journal of Entomology1-3. https://doi.org/10.55446/ije.2022.803
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of

conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87 (6): 651-701. https://doi.org/10.1093/aesa/87.6.651

- Simon E (1914) Les arachnides de France. Synopsis générale et catalogue des espèces françaises de l'ordre des Araneae. Synopsis générale et catalogue des espèces françaises de l'ordre des Araneae VI. 1: 1-308.
- Song DX, Zhu MS, Chen J (1999) The spiders of China. Hebei Science and Technology Publishing House, Shijiazhuang, 64 pp.
- Tahami M, Zamani A, Sadeghi S, Ribera C (2017) A new species of *Loxosceles* Heineken & Lowe, 1832 (Araneae: Sicariidae) from Iranian caves. Zootaxa 4318 (2). https://doi.org/10.11646/zootaxa.4318.2.10
- Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38 (7): 3022-3027. https://doi.org/10.1093/molbev/msab120
- Taucare-Ríos A, Nentwig W, Bizama G, Bustamante RO (2018) Matching global and regional distribution models of the recluse spider *Loxosceles rufescens*: to what extent do these reflect niche conservatism? Medical and Veterinary Entomology 32 (4): 490-496. https://doi.org/10.1111/mve.12311
- Trivedi V, Dal P (2019) Occurrence, distribution and description of *Loxosceles rufescens* (Dufour, 1820) (Araneae: Sicariidae) from western India. Journal of the Bombay Natural History Society 116: 1-8. https://doi.org/10.17087/jbnhs/2019/v116/123017
- Valdez-Mondragón A, Cortez-Roldán M, Juárez-Sánchez A, Solís-Catalán K (2018) A new species of *Loxosceles* Heineken & Lowe (Araneae, Sicariidae), with updated distribution records and biogeographical comments for the species from Mexico, including a new record of *Loxosceles rufescens* (Dufour). ZooKeys 802: 39-66. https://doi.org/10.3897/zookeys.802.28445
- Vetter R (2008) Spiders of the genus *Loxosceles* (Araneae, Sicariidae): a review of biological, medical and psychological aspects regarding envenomations. Journal of Arachnology 36 (1): 150-163. https://doi.org/10.1636/rst08-06.1
- Vetter RS, Visscher PK (1998) Bites and stings of medically important venomous arthropods. International Journal of Dermatology 7: 481-496. https://doi.org/10.1046/j.1365-4362.1998.00455.x
- World Spider Catalog (2023) World Spider Catalog. Version 24. Natural History Museum Bern. http://wsc.nmbe.ch. Accessed on: 2023-3-14.
- Zamani A, Mirshamsi O, Marusik YM (2020) ‘Burning Violin’: The medically important spider genus *Loxosceles* (Araneae: Sicariidae) in Iran, Turkmenistan, and Afghanistan, with two new species. Journal of Medical Entomology 58 (2): 666-675. https://doi.org/10.1093/jme/tjaa257

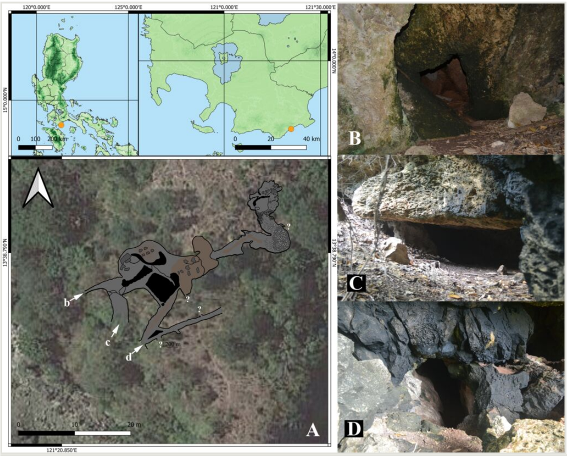

Figure 1.

Map of Kamantigue Cave in Lobo, Batangas, Philippines (**A**) and cave entrances (**B-D**). Cave map by DENR CENRO-Lipa, Batangas; b, c, d = entrance; ? - unexplored areas.

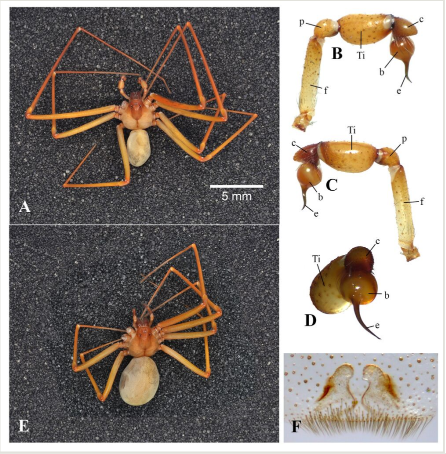

Figure 2.

Loxosceles rufescens (Dufour, 1820): Male (**A-D**) and Female (**E-F**); Habitus, dorsal view (**A, E**); palp: prolateral (**B**), retrolateral (**C**), apical (**D**); spermatheca (**F**). Abbreviations: f - femur, p - patella, Ti - tibia, c - cymbium, b - bulb, e - embolus.

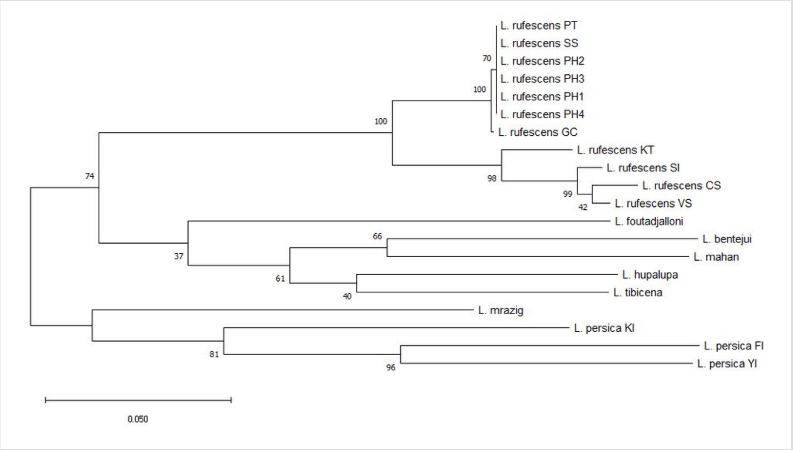

Figure 3.

UPGMA tree generated using the *COI* sequences of the four Philippine *Loxosceles rufescens* (Dufour, 1892) specimens and 16 other *Loxosceles* species. Evolutionary distances were calculated using the Tamura-Nei parameter model with 1,000 bootstrap iterations. CS – Cabo de Gata, Spain; FI – Fars, Iran; GC – Subida San Felipe, Gran Canaria; KI – Khuzestan, Iran; KT – Kepez, Turkey; PH – Batangas, Philippines; PT – Porto Santo, Portugal; SI – Sardinia, Italy; SS – Sagunt, Spain; VS – Vilamarxant, Spain; YI – Yazd, Iran.

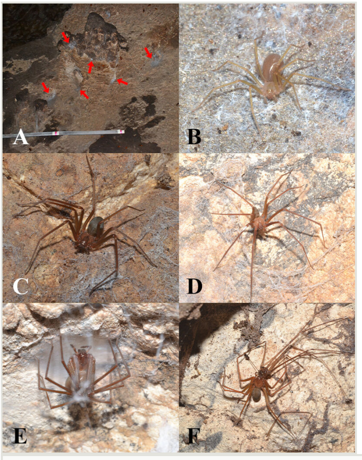

Figure 4.

Loxosceles rufescens (Dufour, 1820) in Kamantigue Cave. **A** web in cave wall; **B** Juvenile; **C** Male; **D** Female; **E** Female with eggs; **F** Female feeding on a pholcid, *Smeringopus pallidus* (Blackwall, 1858).

Table 1.

Localities of *Loxosceles* spp. sequences used for phylogenetic analysis.

Species	Locality	Accession	Reference
L. bentejui	Agaete, Gran Canaria	KF669917	Planas and Ribera (2014)
L. foutadjalloni	Segueya, Guinea	GQ279239	Duncan et al. (2010)
L. hupalupa	Igualero, La Gomera	KF670003	Planas and Ribera (2014)
L. mahan	Teguitar, Fuerteventura	KF669928	Planas and Ribera (2014)
L. mrazig	Douz, Tunisia	FJ986179	Ribera and Planas (2009)
L. persica FI	Kaviri Cave, Ghaemiye, Fars, Iran	MF467576	Tahami et al. (2017)
L. persica KI	Ker Palang Cave, Mal Agha, Khuzestan, Iran	MF467577	Tahami et al. (2017)
L. persica YI	Khaneh Khoda Cave, Harat, Yazd, Iran	MF467575	Tahami et al. (2017)
L. rufescens CS	Cabo de Gata, Spain	KF717002	Planas and Ribera (2014)
L. rufescens GC	Subida San Felipe, Gran Canaria	KF717009	Planas and Ribera 2014
L. rufescens KT	Kepez, Turkey	KJ560582	Planas et al. 2014
L. rufescens PH1	Kamantigue Cave, Lobo, Batangas, Philippines	OR835851	this study
L. rufescens PH2	Kamantigue Cave, Lobo, Batangas, Philippines	OR835852	this study
L. rufescens PH3	Kamantigue Cave, Lobo, Batangas, Philippines	OR835853	this study
L. rufescens PH4	Kamantigue Cave, Lobo, Batangas, Philippines	OR835854	this study
L. rufescens PT	Porto Santo, Portugal	KF717006	Planas and Ribera (2014)
L. rufescens SI	Sardinia, Italy	KJ560589	Planas et al. (2014)
L. rufescens SS	Sagunt, Spain	KF717003	Planas and Ribera (2014)
L. rufescens VS	Vilamarxant, Spain	KF717004	Planas and Ribera (2014)
L. tibicena	Cumbre Arico, Tenerife	KF669925	Planas and Ribera (2014)

Table 2.

Leg measurements of *Loxosceles rufescens* (Dufour, 1820) from the Philippines.

Sex	Leg	Femur	Patella	Tibia	Metatarsus	Tarsus	Total
Male	1	7.25	0.88	8.30	8.65	1.33	26.40
	2	8.95	1.08	9.20	9.25	1.65	30.13
	3	6.55	0.90	7.55	7.45	1.45	23.90
	4	7.10	0.85	7.00	7.50	1.68	24.13
	Pedipalp	1.33	0.28	0.75	-	1.33	3.68
Female	1	5.33	0.91	5.80	5.00	1.09	18.13
	2	5.85	0.96	6.28	5.58	1.18	19.84
	3	4.85	1.00	4.45	4.83	1.05	16.21
	4	5.31	0.95	5.43	6.18	1.13	18.99
	Pedipalp	1.21	0.35	0.83	-	1.21	3.60

Author-formatted, not peer-reviewed document posted on 11/12/2023. DOI: https://doi.org/10.3897/arphapreprints.e117085

Table 3.

Pairwise Distance Matrices of *COI* sequences of *Loxosceles rufescens* (Dufour, 1820) from the Philippines and other localities: CS – Cabo de Gata, Spain; GC – Subida San Felipe, Gran Canaria; KT – Kepez, Turkey; PH – Batangas, Philippines; PT – Porto Santo, Portugal; SI – Sardinia, Italy; SS – Sagunt, Spain; VS – Vilamarxant, Spain.

	CS	**GC**	**KT**	**PH1**	**PH2**	**PH3**	**PH4**	**PT**	**SI**	**SS**	**VS**	
CS		0.0211	0.0132	0.0273	0.0284	0.0274	0.0269	0.0208	0.0072	0.0211	0.0060	**Standard Deviation**
GC	0.0836		0.0211	0.0022	0.0023	0.0023	0.0022	0.0017	0.0208	0.0016	0.0198	
KT	0.0509	0.0843		0.0247	0.0262	0.0254	0.0249	0.0204	0.0128	0.0197	0.0125	
PH1	0.0966	0.0023	0.0875		0.0000	0.0000	0.0000	0.0000	0.0263	0.0000	0.0233	
PH2	0.0989	0.0024	0.0927	0.0000		0.0000	0.0000	0.0000	0.0272	0.0000	0.0240	
PH3	0.0959	0.0024	0.0899	0.0000	0.0000		0.0000	0.0000	0.0264	0.0000	0.0233	
PH4	0.0941	0.0023	0.0882	0.0000	0.0000	0.0000		0.0000	0.0259	0.0000	0.0228	
PT	0.0835	0.0024	0.0825	0.0000	0.0000	0.0000	0.0000		0.0199	0.0000	0.0190	
SI	0.0228	0.0812	0.0493	0.0913	0.0932	0.0904	0.0887	0.0793		0.0210	0.0055	
SS	0.0866	0.0023	0.0803	0.0000	0.0000	0.0000	0.0000	0.0000	0.0832		0.0200	
VS	0.0179	0.0778	0.0479	0.0815	0.0828	0.0803	0.0789	0.0759	0.0157	0.0805		
	Pairwise Distance											