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TECHNICAL NOTE ON THE INTRODUCTION OF PARTRIDGE COLOURED HUNGARIAN CHICKEN IN THE MEKONG DELTA OF VIETNAM

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Abstract

The paper aims to provide a brief agricultural profile of Tra Vinh province, informative adaptation results of Partridge coloured Hungarian chicken (PH) in Mekong Delta and describe the procedure to introduce PH into Tra Vinh province. During the introducing process, flexibility, consideration of the local condition (temperature, humidity, daily sunlight...), and availability of local resources such as bamboo blind, rice husk is essential for introducing a new chicken breed into Mekong Delta. For this, practical examples are given in the study. Regarding adaptation results, relatively high survival rate (89.6%) of PH was recorded at the end of 8th week. Although the recorded data of PH in Tra Vinh is limited, their performance is expected to be equally good or even better in comparison with that obtained in the sub-tropical climatic zone (North Vietnam). Further studies of PH adaptability in Mekong Delta for sustainable, traditional production and crossing purposes, as well as the involvement of chicken caravans to free range farming are recommended.

Key words: chicken caravan, partridge coloured Hungarian, adaptation, Tra Vinh, Vietnam

Összefoglalás

A tanulmány röviden bemutatja Tra Vinh (Vietnam) tartomány agrárgazdaságát, a fogolyszínű magyar tyúk (PH) adaptációs vizsgálatának helyi technológiai feltételeit és eredményeit a Mekong-deltában, Tra Vinh tartományban. Egy új tyúkfajta bevezetése során a rugalmasság, a helyi körülmények (hőmérséklet, páratartalom, nappalhossz) figyelembevétele, valamint a helyi erőforrások (pl. bambusz roló, rizshéj) hasznosítása meghatározó, melyre a tanulmány gyakorlati példákat mutat be. Az adaptációs vizsgálatok során a PH fajtát viszonylag jó életképesség (89.6% túlélési arány) jellemezte 8 hetes életkorig. Bár egyelőre kevés adat áll rendelkezésre a PH fajtáról Tra Vinhben, az előzetes vizsgálatok szerint a termelése nem marad el az Észak-Vietnamban, szubtrópusi körülmények között mért eredményektől. A szerzők további adaptációs vizsgálatokat javasolnak a PH fajta helyi hasznosítására a fenntartható, hagyományos termelésben és keresztezési programokban, kiegészítve a baromfi vándorlók helyi használatával a szabadtartásos tyúktenyésztésben.

Kulcsszavak: baromfi vándorló, fogolyszínű magyar tyúk, adaptáció, Tra Vinh, Vietnam

Introduction

Hungarian chicken breeds, including Partridge coloured Hungarian chicken (PH), are originated from the Hungarian landrace chicken. First reports on breeding special colour varieties as a separate breeds are dated back to the early 1900s (Szalay, 2002). Over the centuries Hungarian chickens adapted well to the climate, keeping condition and farming system of the Carpathian Basin. In spite of its long breeding history, registered *in situ* gene bank stock of PH was established succeeding an effective gene rescue programme of the Research Centre for Farm Animal Gene Conservation (HáGK) not long ago (Szalay et al, 2009; Szalay, 2015). PH, just like all other local Hungarian chickens, were reported to have not only excellent meat quality regardless of hot or cold weather (Baldy, 1954) but relatively good egg producing capability in the continental climate, in which PH is superior to other native Hungarian breeds (Lan Phuong et al, 2014). According to FAO (1992), adapting and maintaining live populations of rare farm animal breeds outside of their native environment are listed as possible *ex situ* conservation methods. It was effectively implemented by various authors in indigenous poultry conservation (Tien et al, 2010, Zanetti et al, 2010; Rusfidra et al, 2015). The Association of Hungarian Small Animal Breeders for Gene Conservation (MGE) and KÁTKI (predecessor of HáGK) had introduced local Hungarian landrace guinea fowl and Hungarian turkey breeds into both subtropical and tropical regions of Vietnam for experimental purposes between 2002 and 2007, cautiously considering the protection of more than 30 native Vietnamese chicken breeds (Lan Phuong et al, 2015) and demands for sustainable agriculture (Szalay and Dong Xuan, 2007). As expected, these breeds successfully adapted and reproduced efficiently (Dong Xuan et al, 2008; Dong Xuan et al, 2015), similar to other exotic chicken breeds such as Luong Phuong chicken of Chinese (Thuan, 2003; Doan and Thanh, 2011) and Fayoumi chicken of Egyptian origin (Nhan et al, 2010; Tuyen et al, 2010). Those adaptation studies suggested that the introduction of PH chicken into Vietnam can also be favourable. In 2011, through transnational collaboration between HáGK and Thuy Phuong Poultry Research Centre (POREC), PH was introduced to Vietnam for the first time. Following the adaptation study of PH in North Vietnam (subtropical climatic zone), MGE had developed a NEFE project with special regards to Poultry Research for Development (PRD) in disadvantageous regions of the Mekong Delta to bring PH to Southwest Vietnam (tropical climatic zone). After a methodical discussion and contact, Tra Vinh province was identified as a new potential breeding region in the Mekong Delta and selected for joining this project. The paper aims to provide a brief agricultural profile of Tra Vinh province, informative adaptation results of Partridge Coloured Hungarian (PH) in the Mekong Delta and describe the procedure to introduce PH into Tra Vinh province.

Agricultural profile of Tra Vinh province

Tra Vinh is located in the Mekong Delta region, which is in the Southern part of Vietnam, bordered by the East Sea to the East with the coastline of 65 km, Vinh Long province to the West, Soc Trang province to the South and Ben Tre province to the North (Tam and Thao, 2004). Out of 1027.5 thousand inhabitants of Tra Vinh, more than 300 thousand belong to the Khmer ethnic group (Lonely Planet, 2009). The province is enclosed by Tien and Hau River, two main branches of Mekong River. The flow of those branches is regulated by their link to Tonle Sap, an inland lake in Cambodia. The lake absorbs any excess flow of water and supplements a reduction in flow by its large reserve storage. Therefore, the environment of the Mekong Delta generally and Tra Vinh particularly is more predictable and benign than that of Red River in the North Vietnam (Jamieson, 1995). Situating in tropical climatic zone, in Tra Vinh, mean air temperature is between 25°C and

28°C; monthly sunshine duration is between 132 and 284 hours; (Cang Long station) and monthly mean humidity is about 78-88%. In dry season (December-April), monthly mean rainfall is less than 90mm, while in rainy season (May-November) it can go up to 260mm (GSO, 2013). It is rarely affected by storm and flood, thus, very favourable for agricultural production. Some statistical data related to agriculture of Tra Vinh province are listed in *Table 1*.

Table 1: Statistical data related to agriculture of Tra Vinh province (GSO, 2013)

Type of data	Unit	Amount
Agricultural production land	thousand ha	148.2
Number of farms	farm	70
Number of livestock farms	farm	19
Number of poultry	thousand heads	5176
Number of buffaloes	thousand heads	1.3
Number of cattle	thousand heads	131.4
Number of pigs	thousand heads	403
Production of aquaculture	tons	88 361
Production of fishery	tons	162 744
Production of cereals per capita	kg	1268.2
Production of paddy	thousand tons	1274.8

Procedure to introduce PH into Tra Vinh province

Day old PH chicks were hatched in POREC and carried to Can Tho city by airplane, and then by mini bus from Can Tho city to Tra Vinh province. The transport of day old chicks from hatchery to farm has a critical role to play in subsequent performance. Hatcheries operate in a fully controlled indoor environment, while transport entails the risk of exposing the chicks to uncontrolled, outdoor conditions. If the chicks are not protected from unpredictable changes to their climate, varying road conditions, traffic jams and other delays, their performance is directly impaired. Prior to departure, all chicks were fed and supplemented with Vitamin C.

500 chicks were allocated in 5 corrugated chick boxes, made from grade raw materials 100birds/box). Each box composed of 4 compartments (25birds/compartiment). Fresh water spinach (*Ipomoea aquatica*) was placed in all boxes as water supplement. *Ipomoea aquatica* is a popular semiaquatic, tropical plant grown as a vegetable for its tender shoots and leaves. It is very rich in water, vitamins A and C. Along the whole journey, transporting environment was optimised to ensure that the birds arrive at the farm in the same condition in which they left the hatchery. In addition to air ventilator supply, on the way from Can Tho to Tra Vinh, chicks were allowed to rest once. During the break, additional clean water was given to the chicks per oral. It was noted that the mini bus was always parked under the shades to avoid direct heat stress. An area selected for keeping PH chicks was solid, easy to clean and had proper sloping ground for water draining. The area and equipment was disinfected 2 weeks before the arrival of chicks.

In the first 15 days, in order to prevent chicks from wind and other environmental disturbances, the floor, side walls and roof of this area was covered by large sheets of strong, water-proof tarpaulin (*Figure 1*).

Figure 1: Water-proof tarpaulin



(photo taken at MYLAN Group)

It is important to install a roof which is easily closed or opened due to unexpected rain as well as wide difference of mean air temperature in early morning and evening compared to that at noon. Roof was opened during the day to facilitate the heat escape and closed during the night to avoid dew. Bulbs and electric cords were prepared in advance for lighting and heating to maintain the optimal air temperature. Heating apparatus as well as thermometer were placed 30-40 cm above the ground. 20 temporary cages (25chicks/pen, about 10-12 chicks/m²) made from bamboo blinds (Figure 2) were set up. Bamboo blind is the perfect choice for making temporary chick pens due to its eco-friendly, cheap, durable, sturdy, but lightweight characteristics. Moreover, it can be found without difficulty in Mekong Delta. It filters the sunlight from outside, cut the sunlight's heat and brightness while still admitting a glow to the interior on a sunny day.

Figure 2: Chick cages made from bamboo blinds and rice husk bedding



(photo taken at MYLAN Group)

When birds were 3 weeks of age, cages were extended so that they had enough space to move freely (5-6 birds/m²). Rice husk (outmost layer which encases and protects the rice grain) was utilized to make bedding (10-15 cm thick), instead of straw or sawdust (common bedding materials in Europe). It not only has low cost but also provide good insulation, neither attract insects nor absorb urine and faeces. More importantly, rice husk is fire resistant and a good soil compost after removal. A thin paper sheet was intentionally placed on the top of rice husk bedding, which could help to reduce leg injuries and keep bedding dry. The paper sheet was changed three times a day. Each cage was provided with sanitized shallow plastic feeder and drinking trough. After 4 weeks of age, birds were moved to permanent wooden pens (8-10 birds/m²). Pens were constructed towards the East. In this way, birds could receive soft sunlight in the morning and stay away from strong sunlight, source of heat in the afternoon. The same material was used to make bedding without paper sheet covering. 2 perches were installed 0.5m above the ground floor in each pen. Dead bamboo branches make perfect perches for resting birds and are good places to hang feeders. They were closed at night and let out to graze on fenced pastures (3-4 m²/bird) with some shades during day time. Pasture was positioned parallel with the pens on both sides, in front and at the back. This arrangement made rotating grazing possible, and pasture would have enough time to recover. The pasture was flat, well-drained without stagnant water and foreign objects. In this free range area, feeder and drinking trough were also provided (*Figure 3*).

Figure 3: Free range pasture



(photo taken at MYLAN Group)

In addition to permanent wooden pens, chicken caravan based on a model that won the Australian Farm Invention of the Year 2012 was also built for this experiment. The original model, designed by Australian commercial producer, is very costly. Nonetheless, their idea is promising. Considering potential role of chicken caravan (suggested by MYLAN group) in expanding PH production, NEFE with the help of MYLAN Group, aimed to develop a simplified model that required low investment and fit in small scaled family farming, a traditional but popular chicken keeping system in the Mekong Delta. It composed of 6 pull out shelter doors, two stainless steel drinkers, collector of rainwater off the roof and nesting boxes. Aluminium with lower price and

lighter weight was used to construct the caravan instead of stainless steel. It is strong enough, easy to clean and disinfect. The design of project model emphasised mobile characteristic of the caravan rather than its infrastructure. Wheels that attached to the caravan made it possible to move it from one pasture to another. Dark mosquitos' nets were inserted into the main compartment. It helps to filter sunlight and create an extra shadow when the metal pull-out shades are open (*Figure 4*).

Figure 4: Simplified chicken caravan developed by MYLAN Group



(photo taken at MYLAN Group)

Birds were fed ad libitum and clean water was always available. *Tables 2, 3 and 4 illustrate* a proposed lighting programme, diet (commercial feed) and prophylactic measures applied in this process.

Table 2: Proposed lighting programme

Age (days)	Lighting duration (hours)	Light intensity (W/m ²)
1 – 2	22	5
3 – 4	20	5
5 – 7	17	5
8 – 10	14	3
11 – 13	11	3
14 – 28	8	2
>28	natural sunlight	-

Table 3: Proposed diet

Composition	Age (weeks)			
	0-3	4-7	8-20	21-64
ME (kcal/kg feed)	3000	3000	3100	3100
Crude protein (%)	23	21	18	16
Fibre (% dry matter)	4	5	6	7
Ca (% dry matter)	0.9-1.0	0.9-1.0	1.1-1.3	3.5-4.0
P (% dry matter)	0.4	0.4	0.35	0.40
Lysine (% dry matter)	0.5	0.5	0.5	0.5
Methionine (% dry matter)	0.9-1.0	0.9-1.0	0.8	0.7
Ca (% dry matter)	0.6	0.6	0.4	0.35-0.4

Table 4: Prophylactic measures

Age (days)	Diseases	Vaccine/antibiotics	Route of administration
1	Marek disease	Marek	subcutaneous
1-3	E. coli, Salmonella infection	Enro-flox 5% (2g/l water)	Per oral
5	Newcastle disease	ND-IB	Eyes drop, nasal drop
7	Gumboro disease	Gum B	Eyes drop, nasal drop
11-13	Chronic respiratory disease	D.T.C Vit (2g/l water)	Per oral
14	Gumboro disease	Gum B	Eyes drop, nasal drop
15	Avian Influenza	Nobilis Influenza H5	Subcutaneous injection (neck skin)
19	Newcastle disease	ND-IB	Eyes drop, nasal drop
21	Gumboro disease	Gum B	Eyes drop, nasal drop, per oral
26-28	Coccidiosis	Caticoc-pharm (1g/3l water)	Per oral
40	Newcastle disease	ND-Emulsion	Subcutaneous injection (neck skin)
45	Avian Influenza	Nobilis Influenza H5	Subcutaneous injection (neck skin)

Cleaning and disinfection methods

Since disinfectants would lose effectiveness during contact with organic materials such as manure, blood, dust or dirt, cleaning had been done first in two steps, dry and wet. Broom, brush and shovel were used to remove dust, soil and dry organic material. Then, the area was scrubbed with detergent to eliminate the remaining dirt and grease. A multiple-purpose disinfectant contains potassium peroxymonosulfate, sodium dodecylbenzenesulfonate, sulfamic acid, and inorganic buffers was used to decontaminate surfaces and soak equipment before use.

Informative results of the introduction

At the end of 8th week, 89.6% survival rate, 758 g average body weight and feed conversion ratio of 2.3 kg feed/kg body weight gain were recorded. *Table 5* shows weekly recorded data of PH.

Table 5. Weekly recorded data of Partridge Coloured Hungarian chicken in Tra Vinh province (data provided by Tra Vinh University)

Traits	Weeks of age							
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8
Survival rate (%)	100	100	100	99	100	100	100	100
Body weight (g)	35	60	93	162	251	369	486	758
Feed consumption (g/bird/week)	61	86	157	161	242	297	329	375
Feed conversion ratio (kg feed/kg body weight gain)	-	2.5	2.6	2.3	-	2.5	2.5	2.3

Conclusion and discussion

It is worth noting that flexibility, consideration of the local conditions (temperature, humidity, daily sunlight, local feed etc.) and availability of local resources such as bamboo blind, rice husk is essential for introducing a new free range chicken breed into the Mekong Delta. Relatively high survival rate confirmed the adaptation potential of PH chicken to tropical climatic zone of Vietnam (Tra Vinh province). Although the recorded data of PH in Tra Vinh are limited, with regard to the former results found in guinea-fowl and turkey taken to Vietnam as old Hungarian poultry breeds for adaptation studies (*Dong Xuan et al*, 2008), performance of PH is expected to be equally good or even better in comparison with that obtained in the sub-tropical climatic zone (North Vietnam). Considering conservation of local chicken breeds, PH is recommended to be bred and propagated in a close system (*Szalay and Dong Xuan*, 2007). Further studies on PH adaptability in the Mekong Delta for sustainable, traditional production and crossing purposes, as described by *Dong Xuan et al*, (2006) as well as on the introduction of chicken caravans in free range farming are suggested.

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A new *Loureedia* species on overgrazed former cork oak forest in Morocco (Araneae: Eresidae)

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Abstract

In this paper a new velvet spider species from Morocco is described from an overgrazed former cork oak [*Quercus suber* (Linné 1753)] forest. It is the second known species of the hitherto monotypic genus *Loureedia*. *Loureedia maroccana* **sp. n.** is distinguished from *L. annulipes* (Lucas, 1857) by the morphology of the conductor, the anteriorly widening cephalic region of the prosoma and opisthosoma decorated with a lobed, bright red marking on the dorsal side. Furthermore, three partial gene fragment sequences (histone 3, 28S ribosomal and cytochrome c oxidase) are also given, supporting the establishment of the new species.

Keywords: *Loureedia*, velvet spiders, cork oak, Morocco

Introduction

Velvet spiders (Eresidae) contains nine genera and 96 described species worldwide (*World Spider Catalog* 2017). According to the present knowledge, the monotypic genus *Loureedia* was established by Miller et al, (2012) based on *L. annulipes*, the type species, which described in Israel. Former publications mentioned two synonyms of *L. annulipes*: *Eresus semicanus* Simon, 1908 and *Eresus jerbae* El-Hennawy, 2005 (Simon 1908; El-Hennawy 2005).

Loureedia annulipes was originally described as *Eresus annulipes* Lucas, 1857. The genus *Loureedia* mainly differs from the other velvet spider genera in having a strongly bifid apical region of the conductor, in the shape of the cephalic region of the prosoma and also in the extremely bright pattern of the dorsal side of the opisthosoma. At present, *L. annulipes* is known from Algeria, Tunisia, Egypt, Israel (Miller et al, 2012) and Spain (Nentwig et al, 2017).

Zakkak et al, (2014) found a positive correlation between the ground spider richness and low intensity grazing. Horváth et al, (2013) found that the spiders are less diverse in overgrazed grasslands and the negative effect is minimal in small and isolated grasslands.

In this paper, we present a species belonging to the hitherto monotypic genus *Loureedia*, collected in an overgrazed cork oak forest in Morocco. Thorough examination of these specimens showed coherent morphological characteristics clearly different from those of *L. annulipes*, and the species is described here as new to science.

Materials and methods

Specimens were collected individually and stored in 70 % ethyl-alcohol. Three males and the palps of one additional specimen partially destroyed during transportation were studied. All the measurements are given in millimetres (mm).

The holotype and paratypes have been deposited in the Soil Zoological Collection (former Arachnoidea Collection) of the Department of Zoology, Hungarian Natural History Museum (collection number of holotype: HNHN Araneae-8869 and collection number of paratype: HNHN Araneae-9007) Budapest (curator Dr. László Dányi).

Specimens and copulatory organs were studied using a Leica MZ FL III stereomicroscope and photographed by Canon Q Imaging Micro 5.0 RTV at the Institute of Genetics, BRC. Scanning electron micrographs were taken with a Hitachi S-4700 microscope at the Department of Applied and Environmental Chemistry, University of Szeged, Hungary.

One segment of a spider leg was used to extract total genomic DNA after the modified *Drosophila* DNA extraction protocol (Engels et al. 1990). One µl of extracted DNA was used as template in the total amount of 25 µl polymerase chain reaction (PCR) following the manufacturer's instructions (Promega GoTaq® Hot Start Kit). Reactions were conducted with two set of nuclear primers (for histone 3-H3 and 28S rRNA partial genes) and one set of mitochondrial primer pair (for cytochrome c oxidase subunit I – COX1 partial gene). Primer sequences are listed in Supplementary file, *Table 1*. PCR products were controlled on agarose gel and purified after gel electrophoresis following the manufacturer's protocol (Zymoclean™ Gel DNA Recovery Kit) and were sequenced by Macrogen Inc.

Raw sequences were assembled in Staden Package 2.0 (Staden et al, 2000). Each base call and any discrepancies of the sequences were corrected according base confidence values (Bonfield et al, 2010). Sequences used in this study were obtained from GenBank with the accession numbers shown in supplementary material (see *Table 2*). Accession numbers of the newly sequenced taxa are the following: *Loureedia maroccana* sp. n. isolate LIV, KX443580 (28S rDNA), KX443586 (H3), KX443583 (COX1); *Eresus* sp. isolate C4d, KX443581 (28S rDNA), KX443587 (H3), KX443584 (COX1); *Eresus sandaliatus* isolate JL-1589, KX443582 (28S rDNA), KX443588 (H3), KX443585 (COX1).

Consensus sequences were aligned using the MUSCLE (Edgar 2004) algorithm in MEGA 6.06 (Tamura 2013). The alignment was further curated in BioEdit 7.0.9.0 (Hall 1999). The genetic distances between taxa were assessed by MEGA 6.06.

Table 1: List of primer pairs used in this study

Primer		Sequence	Reference
Forward	LC01490-oono	CWA CAA AYC ATA RRG ATA TTG G	Modified from Folmer et al. (1994) in Miller et al. (2010)
Reverse	HC02198	TAA ACT TCA GGG TGA CCA AAA AAT CA	Folmer et al. (1994)
Forward	H3nF	ATG GCT CGT ACC AAG CAG AC	Colgan et al. (1998)
Reverse	H3aR	ATA TCC TTR GGC ATR ATR GTG AC	Colgan et al. (1998)
Forward	28S0	GAA ACT GCT CAA AGG TAA ACG G	Hedin and Maddison (2001)
Reverse	28SC	GGT TCG ATT AGT CTT TCG CC	Hedin and Maddison (2001)

Table 2: GenBank accession numbers obtained from GenBank. New sequences generated for this study are shown in bold.

Species	Code	28S	H3	COI
<i>Eresus cf. kollari</i> 14_04	14_04	FJ948958	FJ949036	FJ948998
<i>Eresus sandaliatus</i>	JL-1589	KX443582	KX443588	KX443585
<i>Eresus</i> sp. 13_06	13_06	FJ948957	FJ949035	FJ948997
<i>Eresus</i> sp. C4d	C4d	KX443581	KX443587	KX443584
<i>Eresus walckenaeri</i> 14_05	14_05	FJ948959	FJ949037	FJ948999
<i>Gandanameno fumosa</i> 09_05	09_05	FJ948963	FJ949041	FJ949003
<i>Gandanameno fumosa</i> 14_6	14_06	FJ948964	FJ949042	FJ949004
<i>Gandanameno</i> sp. 09_02	09_02	FJ948962	FJ949040	FJ949002
<i>Gandanameno</i> sp. 13_10	13_10	FJ948961	FJ949039	FJ949001
<i>Loureedia</i> (former <i>Stegodyphus</i>) <i>annulipes</i> 15_10	15_10	FJ948960	FJ949038	FJ949000
<i>Loureedia maroccana</i> sp. n. LIV	LIV	KX443580	KX443586	KX443583
<i>Paradonea variegata</i>	14522	-	-	JQ026517
<i>Paradonea variegata</i>	14512	JQ026518	-	JQ026516
<i>Stegodyphus lineatus</i> 14_02	14_02	FJ948976	FJ949053	FJ949016
<i>Stegodyphus mimosarum</i> 09_06	09_06	FJ948977	FJ949054	FJ949017
<i>Stegodyphus tentoriicola</i> 14_12	14_12	FJ948975	FJ949052	FJ949015

Abbreviations

Standard abbreviations of morphological terms follow *Miller et al.*, (2012). Further abbreviations: **PME** = posterior median eyes, **PLE** = posterior lateral eyes.

BRC Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary;

HNHM Hungarian Natural History Museum, Budapest, Hungary;

Results and discussion

Taxonomy

Loureedia maroccana sp. n.

Material examined. Holotype: Male. Morocco, near the locality of Sidi Boukhalkhal, N 34° 05' 57,70'', W 6°24' 23,22'', singled, 04.11.2013., J. Gál (HNHM, collection number: HNHM Araneae-8869).

Paratypes: 2 Males. Morocco, close to Sidi Boukhalkhal, N 34°07'16,65'', W 6°25'36,44'', singled, 28.10.2015., R. Bagyó (HNHM, collection number: HNHM Araneae-9007)

Etymology. The species is named after the country of the type locality, Morocco.

Generic placement. This species has a wider than long cephalic region (*Fig. 1*), a median eye group with the PME clearly larger than the AME, it lacks tubercles associated with ALE, has a palpal conformation with a proximal-distal axis, a helical embolus encircling the distal part, and a strongly bifid (doubly pronged) conductor. These features together unambiguously place this species within the heretofore monotypic genus *Loureedia*.

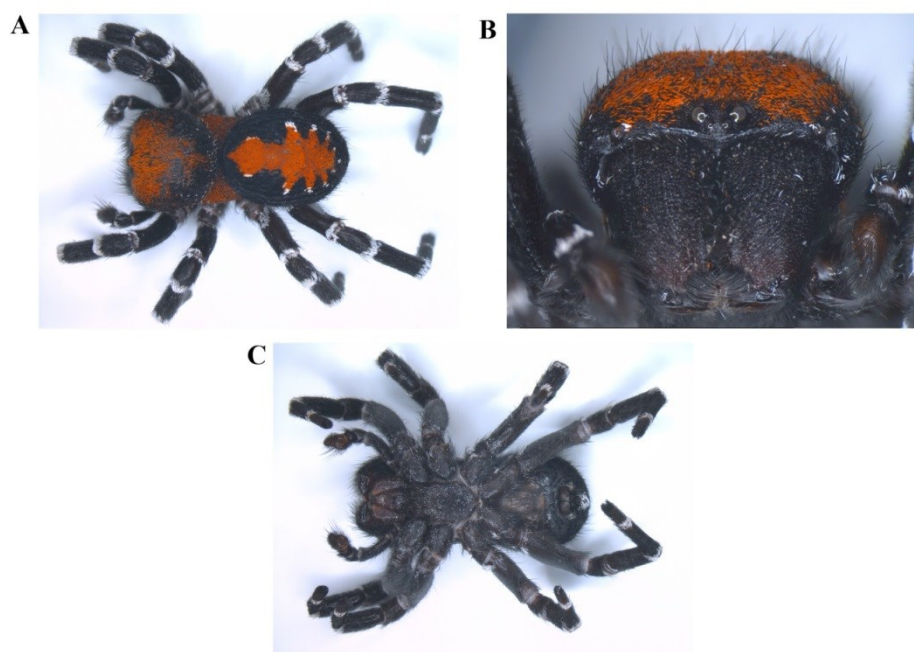
Figure 1: Habitus of living adult male specimen of *Loureedia maroccana* sp. n. (HNHM, collection number: HNHM Araneae-8869)



Diagnosis. Distinguished from males of the only other member of the genus *Loureedia*, *L. annulipes*, by the cephalic region, which is subtrapezoidal when viewed from above, clearly bulging laterally with doubly arched lower margin above chelicerae in frontal view; the clypeal hood, which is acutely angled with concave sides and the apical palpal complex with embolic division longer than tegular division. By contrast, *L. annulipes* males are characterized by a cephalic region with subrectangular outline when viewed from above, with nearly parallel sides and almost flat lower margin at the base of chelicerae in frontal view; clypeal hood forming a nearly 90° angle with strait sides and an apical palpal complex with embolic division shorter than tegular division. In addition, the edge of the dorsal prong of the conductor is evenly curved in the case of the *L. maroccana* while it is clearly S-shaped in *L. annulipes* (shown by Miller et al, 2012). Carapace and opisthosoma of *L. maroccana* are predominantly black and red, as opposed to the variable, but usually white-decorated (often in combination with orange yellow) body of *L. annulipes*.

Description. Male. Prosoma (Fig. 2): Lengths: 4.5; 3.95; 3.1. Carapace dark blackish brown, cephalic region dorsally covered by short red setae on the front and the centre, with some scattered red hairs on the flanks, scattered white hairs restricted to the posterior and to the extreme anterior edge; remaining area covered by black setae. Carapace covered by red setae, except for a short longitudinal, black bar running through the moderately deep fovea, and a dark blackish-brown posterior triangle mostly devoid of hairs. Cephalic region steeply ascending posteriorly, then evenly rounded until about PLE, followed by a region gradually decreasing towards PME. AME distinctly smaller than PME, ALE not associated with tubercle. Viewed from above, cephalic part somewhat wider than thoracic part, clearly wider than long, subtrapezoidal, widening towards anterior third; posteriorly arcuate, broadly rounded laterally, and with a shallow, longitudinal depression along the midline most obvious at the posterior third. In frontal view, lower margin of carapace arched above the articulation of each chelicera, flanks slightly, but clearly bulging laterally. Clypeal hood acute-angled is with slightly concave sides.

Figure 2: A-C. Habitus of adult male specimen of *Loureedia maroccana* sp. n.: A. dorsal view, B. frontal view, C. ventral view. (HNHM, collection number: HNHM Araneae-9007).



Chelicerae (Fig. 2): black, covered by long, nearly adpressed black hairs.

Legs and palps (Figs. 2 and 3-4): black to dark grey, white striped dorsally at joints. Palps with a proximal-distal axis, apical complex making slightly more than one helical turn. Embolic division somewhat longer than tegular division, membranous conductor abruptly transitioning just before a deep cleft dividing the conductor dorsally-retrolaterally into a heavily sclerotized, two-pronged structure with the dorsal prong flatly and evenly curved at the edge facing the cleft.

Opisthosoma (Figs. 1, 2): dark blackish brown, covered by black/dark grey setae, decorated with a narrow crescent covered by white hairs at the lower anterior edge and with a roughly almond-shaped red area along the dorsal midline with white-tipped lateral lobes. In contrast *L. annulipes* (see Miller et al, 2012); *L. maroccana* possesses a fig leaf shaped dorsal colour pattern of fire red colour. It lacks a dark medieval centre line.

Remark. One of the collected specimens lacks white spots at the tips of the anterior-most pair of lateral lobes.

Female: unknown.

Figure 3: A-C. Photomicrographs of *Loureedia maroccana* sp. n. male right palp: A. prolateral view, B. ventral view; C. retrolateral view.

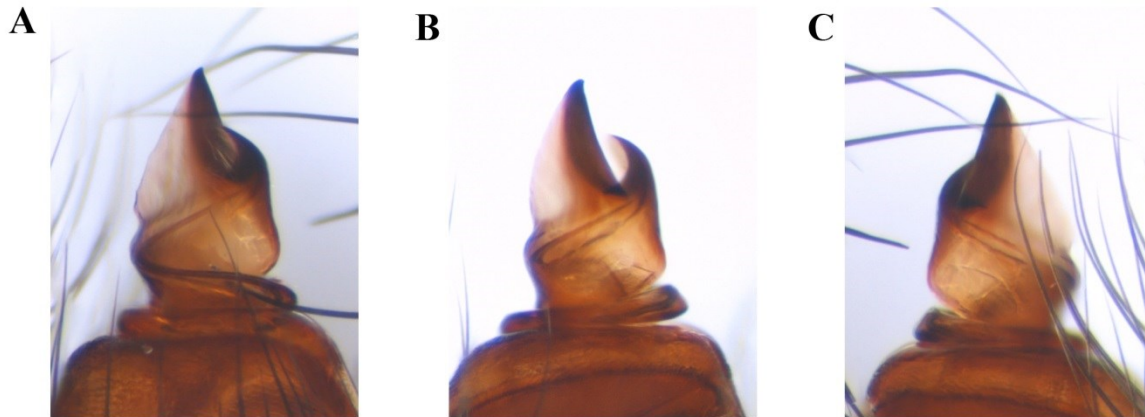
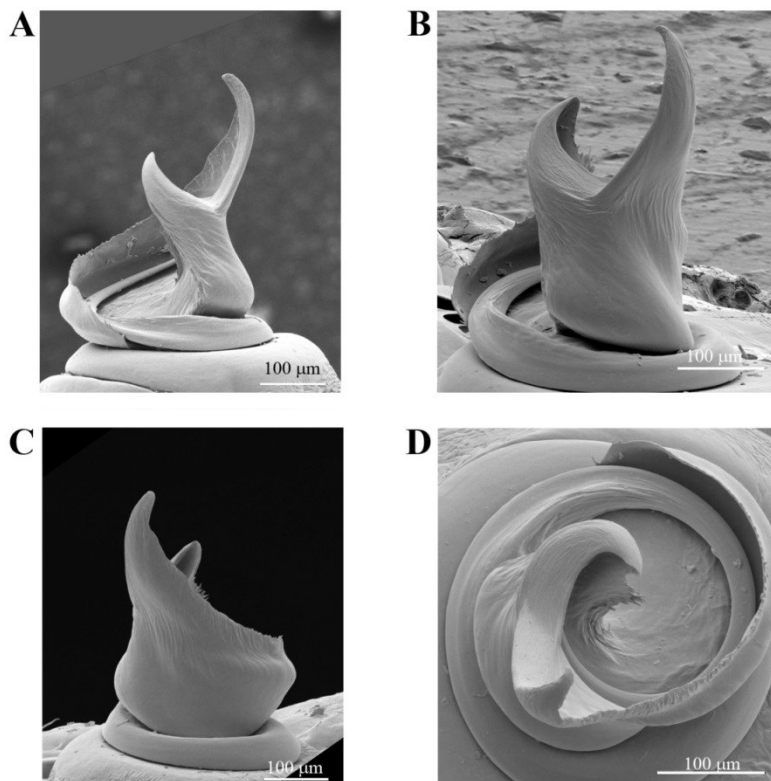


Figure 4: A-D. Scanning electron micrographs of *Loureedia maroccana* sp. n. adult male left palp: A. prolateral view, B. ventral view, C. retrolateral view, D. apical view.



Distribution. At the time of manuscript submission known only from the type locality, close to Sidi Boukhalkhal in overgrazed former cork oak forest.

Habitat. Collected specimens have been found from glades of semi-natural *Q. suber* woods on the southern dry slopes of the western foothills of the Moroccan Middle Atlas Mts. The habitat was strongly overgrazed by sheep and goat.

Phenology. Males were found wandering on the surface of soil between September and November, indicating a late autumnal copulation period.

Note. The finding that males of *L. maroccana* have a subtrapezoidal cephalic region requires a slight modification of the circumscription of the genus *Loureedia*, as the subrectangular shape of cephalic region can no longer be considered as a distinguishing character. However, this in no way affects the stability of the genus, since numerous other characters (see Miller et al, 2012) set *Loureedia* apart from the other genera of family Eresidae.

Genetic examination

318, 399 and 725 base pair long partial gene fragments were obtained by H3, COX1, and 28S primer pairs respectively. The mitochondrial sequences differ by 10.27 % between *L. annulipes* and *L. maroccana* specimens, similarly to other interspecific sequence divergence estimates of mitochondrial markers among Eresidae (Johannesen et al, 2005; Johannesen et al, 2007; Robinson et al, 2009). The sequence diversity of 28S rRNA nuclear gene fragment is 1.2 % between the two *Loureedia* species. The variability of 28S rRNA gene fragment between these species is higher than the average interspecific sequence divergence among the examined *Eresus* species, which is 0.7 %. The H3 gene fragments of the two *Loureedia* species were compared and no gaps were found, but sequence polymorphisms were identified at 12 different positions (see the alignment of supplementary material).

Table 3. shows estimates of evolutionary divergence over partial COX1 sequence pairs for intra- and intergeneric level (within and between groups) of some Eresidae genera. The estimates of average genetic distances within the genera were lower than between the examined genera, as expected. The average genetic distance detected between the genera *Loureedia* and *Eresus* is low (0.157), which confirms the findings of Miller et al, (2010) in that the genus *Loureedia* (as *Stegodyphus annulipes*) together with genera *Stegodyphus* constitute a sister group of the *Eresus* clade.

Table 3: Estimates of Average Evolutionary Divergence over Sequence Pairs of partial COX1 gene at intra-and intergeneric level.

A			B				
Taxon name	d.	S.E.	<i>Paradonea</i>	<i>Gandanameno</i>	<i>Loureedia</i>	<i>Eresus</i>	<i>Stegodyphus</i>
<i>Paradonea</i>	0	0		0.027	0.024	0.02	0.024
<i>Gandanameno</i>	0.075	0.013	0.23		0.022	0.019	0.02
<i>Loureedia</i>	0.13	0.023	0.188	0.189		0.016	0.018
<i>Eresus</i>	0.101	0.012	0.163	0.183	0.157		0.015
<i>Stegodyphus</i>	0.143	0.018	0.195	0.193	0.173	0.154	

The average number of base substitutions per site for each sequence pairs (d.) within a given genus (A) and between genera are given (B). Standard error estimates (S.E.) are shown above the diagonal on part B. Analyses were conducted using the LogDet model (Lockhart et al. 1994). The analysis involved 16 nucleotide sequences. All positions with less than 95 % site coverage were

eliminated. A total of 399 positions were retained in the final dataset. The analysis was conducted in MEGA6 (Tamura et al, 2013).

It is worth noting that one change of the 309 position in the COX1 DNA alignment results in the alteration of a predicted Ser of *L. annulipes* into a predicted Lys in *L. maroccana* using ‘in silico’ translated (Stothard 2000) COX1 protein sequences (see the amino acid alignment of supplementary material), also supporting the notion that *L. maroccana* and *L. annulipes* are distinct species.

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