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DNA barcoding the smaller arachnid orders from ACP Panguana, Amazonian Peru

(Amblypygi, Phrynidae and Schizomida, Hubbardiidae)

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Amblypygi and Schizomida were collected at the private protected area ACP Panguana, located in the primary evergreen lowland rainforest of Amazonian Peru. Through integrative taxonomy, using COI barcoding and morphological determination, all amblypygids could be identified as *Heterophrynus elaphus* Pocock, 1903, and the schizomids as *Surazomus chavin* Pinto-da-Rocha, 1996. COI p-distances are provided and DNA sequences were uploaded to BOLD.

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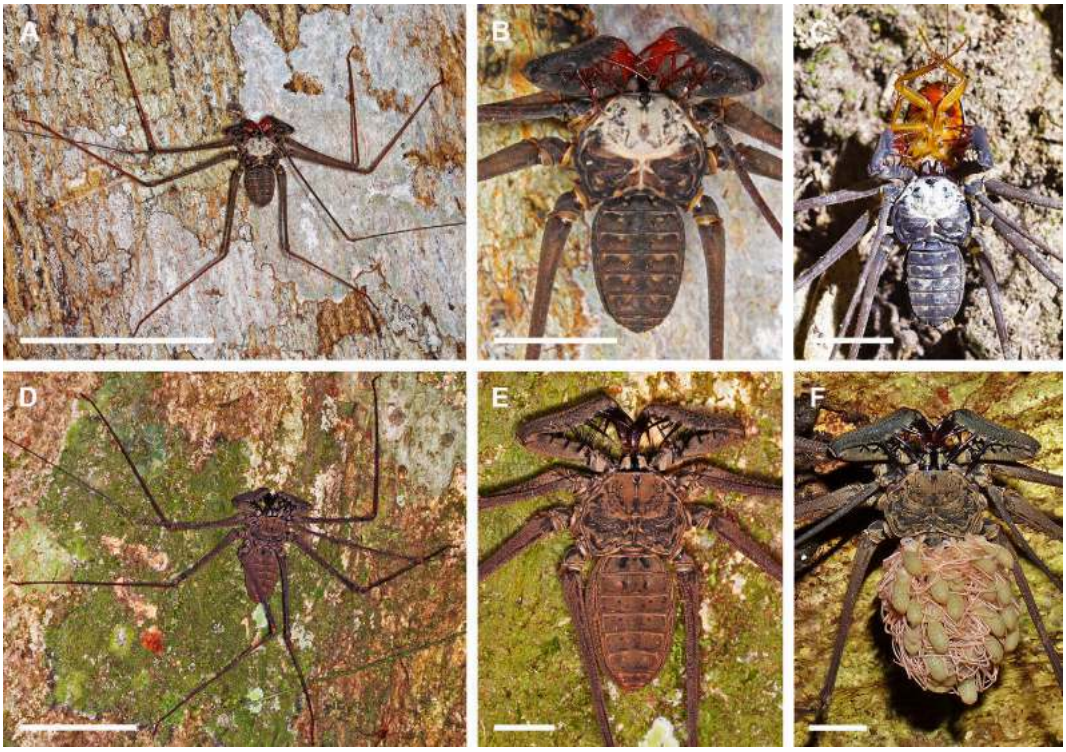
Introduction

Amblypygi, Thelyphonida, Schizomida, Palpigradi, Ricinulei and Solifugae are often combined as the "Smaller Arachnid Orders" (Harvey 2003, Harvey 2007). Recently an inventory of the soil arthropod fauna of the biological field station and private protected area ACP Panguana (Peru, Dept. Huánuco, Rio Yuyapichis, 9°37' S, 74°56' W, 230 m a.s.l.) with a focus on mites and scorpions has been realised (e. g. Kovařík et al. 2015, Ermilov et al. 2016, Kontschán & Friedrich 2017). As a by-product, special attention was paid to these smaller arachnid orders. So far, several specimens of Schizomida and Amblypygi have been collected in the area of Panguana. The schizomids could be collected through sieving upper soil layer and leaf litter with subsequent Winkler extraction (Friedrich 2014). The amblypygids were collected by hand, particularly at the banks of riverbeds and on large trees. Here, two different colour morphs were found: one large generally brownish one and one smaller one with a whitish front part of the prosoma and reddish pedipalps. The smaller specimens always showed the white and red colouration and the larger specimens were generally brown, no intermediate morphs were found. At first sight in the field, we were not sure whether these two morphs are two different species or whether the smaller ones are the juveniles of the larger ones. To solve this issue we used integrative taxonomy (Dayrat 2005, Padial et al. 2010, Schlick-Steiner et al. 2010), which combines morphological species determination by means of classical taxonomy with modern methodological developments like COI DNA barcoding, which helps us to recheck species boundaries or search for cryptic species previously undetected by morphological analysis. Furthermore, one schizomid specimen was DNA barcoded.



Figs A–F. *Heterophrynus elaphus* Pocock, 1903. **A.** Juvenile of *H. elaphus* on a trunk; scale bar = 5 cm. **B.** Detail of juvenile, showing reddish inner side of pedipalps and whitish front part of the prosoma; scale bar = 1 cm. **C.** Juvenile feeding on a cockroach; scale bar = 1 cm. **D.** Adult of *H. elaphus* on buttress root; scale bar = 5 cm. **E.** Detail of adult showing general brownish appearance; scale bar = 1 cm. **F.** Female adult with praenymphae; scale bar = 1 cm.

Material and methods

All specimens were collected from: South America, Amazonian Peru, 09°37'S, 74°56'W, Huánuco Department, Puerto Inca Province, Yuyapichis District, Área de Conservación Privada (ACP) Panguana (biological field station), near Rio Yuyapichis 230 m a.s.l.

Amblypygi: 14 specimens (3 larger and 11 smaller specimens), collected at night by hand, particularly in riverbeds with laterite soil and on trunks and buttress roots of large trees (e.g. *Ceiba pentandra* (Malvaceae), *Ficus* sp. (Moraceae), or *Dipteryx* sp. (Fabaceae)), 2013–2017, leg. S. Friedrich, T. Lehmann, D. Hauth, E.-G. Burmeister & F. Wachtel.

Schizomida: 6 specimens (topotypes), from upper soil and leaf litter in a primary evergreen lowland rainforest by Winkler extraction, 2013–2017, leg. S. Friedrich, D. Hauth & F. Wachtel.

Table 1. Overview of collection data and registration of DNA barcoded specimens.

Museum voucher ID	Species	Country/Region
ZSMA20180002	*Heterophrynus elaphus* Pocock, 1903	Peru, Dept. Huánuco, Rio Yuyapichis, ACP Panguana
ZSMA20180003	*Heterophrynus elaphus* Pocock, 1903	Peru, Dept. Huánuco, Rio Yuyapichis, ACP Panguana
ZSMA20180004	*Heterophrynus elaphus* Pocock, 1903	Peru, Dept. Huánuco, Rio Yuyapichis, ACP Panguana
ZSMA20180005	*Heterophrynus elaphus* Pocock, 1903	Peru, Dept. Huánuco, Rio Yuyapichis, ACP Panguana
ZSMA20180006	*Heterophrynus elaphus* Pocock, 1903	Peru, Dept. Huánuco, Rio Yuyapichis, ACP Panguana
ZSMA20180007	*Heterophrynus elaphus* Pocock, 1903	Peru, Dept. Huánuco, Rio Yuyapichis, ACP Panguana
ZSMA20180008	*Heterophrynus elaphus* Pocock, 1903	Peru, Dept. Huánuco, Rio Yuyapichis, ACP Panguana
ZSMA20180009	*Surazomus chavin* Pinto-da-Rocha, 1996	Peru, Dept. Huánuco, Rio Yuyapichis, ACP Panguana

DNA extraction, amplification and sequencing. Laboratory operations were carried out by AIM – Advanced Identification Methods GmbH in Munich. Depending on the size of the individuals, either whole legs (Schizomida) or a part of a leg (Amblypygi) with muscle tissue from each specimen was taken for DNA extraction and further sequencing. For the DNA COI barcoding (HCOJJ/LCOJJ), failure tracking with dgHCO/dgLCO was used. One schizomid and seven amblypygid specimens were barcoded successfully. Sequences were edited in Sequencher and aligned in MEGA (Kumar et al. 2016). P-distances were analysed in MEGA (Tamura et al. 2004). Specimen data and DNA sequences of the studied species are available from BOLD (Ratnasingham & Hebert 2007) (see Table 1).

Results and discussion

Amblypygi

The figure shows the two colour morphs found in Panguana. Detailed morphological analyses, using Weygoldt (2000), Weygoldt (2002) and Harvey (2003) for determination, showed that these two morphs are indeed the same species, namely *Heterophrynus elaphus* Pocock, 1903. *H. elaphus* used to be considered as endemic for Peru but was recently also recorded for Bolivia (Réveillion et al. 2014). The smaller specimens (Fig. A, B, C) represent the juveniles, which lose their white colouration of the front area of the prosoma and their red colouration of the inner side of the pedipalps during development. The larger specimens (Fig. D, E, F) represent the adults of the same species.

The molecular results affirm the conclusion of the morphological analyses. The genetic divergence between the seven sequenced specimens range between 0.0 %, and 0.8 % (average 0.2 %), what confirms that it is only one species, i.e. *Heterophrynus elaphus* (Table 2). Usually, about 3 % divergence between two sister species is expected (Hendrich et al. 2010). A comparison with *Heterophrynus alces* and *H. longicornis* listed in BOLD showed relatively high p-distances (between 22.9 % and 26.3 %) for species within the same genus (Table 3).

Schizomida

Morphologically the specimens were determined as *Surazomus chavin* Pinto-da-Rocha, 1996. This species has recently been rediscovered at the type locality in Panguana, Dept. Huánuco, Peru, after nearly three decades (Pinto-da-Rocha 1996, Friedrich 2014). Analysis of original and new material showed emendations concerning some morphological features, e.g. the number of articles and the positions of setae on the female flagellum (Friedrich 2014).

A comparison with *Surazomus brasiliensis* and *S. manaus* listed in BOLD showed again relatively high p-distances between 9.2 % and 20.0 % (Table 4).

Latitude	Longitude	Date	COI bp	BOLD ID
09°37'S	74°56'E	20.09.–07.10.2013	617	AIMEH015-18
09°37'S	74°56'E	01.–21.05.2015	553	AIMEH016-18
09°37'S	74°56'E	01.–21.05.2015	251	AIMEH017-18
09°37'S	74°56'E	01.–21.05.2015	617	AIMEH018-18
09°37'S	74°56'E	01.–21.05.2015	609	AIMEH019-18
09°37'S	74°56'E	22.09.–10.10.2017	570	AIMEH020-18
09°37'S	74°56'E	22.09.–10.10.2017	573	AIMEH021-18
09°37'S	74°56'E	22.09.–10.10.2017	197	AIMEH022-18

Table 2. COI p-distances for *Heterophrynus elaphus* specimens from this study.

	1.	2.	3.	4.	5.	6.	7.
1. ZSMA20180002	–						
2. ZSMA20180003	0.000	–					
3. ZSMA20180004	0.000	0.000	–				
4. ZSMA20180005	0.004	0.004	0.004	–			
5. ZSMA20180006	0.004	0.004	0.004	0.008	–		
6. ZSMA20180007	0.000	0.000	0.000	0.004	0.004	–	
7. ZSMA20180008	0.000	0.000	0.000	0.004	0.004	0.000	–

Table 3. COI p-distances for a *Heterophrynus elaphus* specimen from this study and *Heterophrynus* specimens listed in BOLD.

	1.	2.	3.
1. *H. elaphus* ZSMA20180002	–		
2. *H. alces* GACAM006-13	0.249	–	
3. *H. longicornis* GACAM005-13	0.263	0.229	–

Table 4. COI p-distances for a *Surazomus chavin* specimen from this study and *Surazomus* specimens listed in BOLD.

	1.	2.	3.	4.
1. *S. chavin* ZSMA20180009	–			
2. *S. brasiliensis* GBMIN117546-17	0.160	–		
3. *S. manaus* GBMIN117545-17	0.189	0.194	–	
4. *S.* sp. GBMIN117548-17	0.174	0.200	0.092	–

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