Supplementary Materials and Methods

The material analyzed in this study originated from eight sites in French Guiana and Brazil (Table 1, Figure 1), totaling 65 individuals ascribed to three nominal morphospecies, i.e., *H. alces* (6), *H. batesii* (4), and *H. longicornis* (55) (Table 1, Figure 1). Samples were either newly collected material as part of the present study (63 individuals) or were already available from GenBank (two sequences) as part of research conducted by Arabi et al. (2012) on Amblypygi phylogeny. Newly captured animals were ascribed to a given nominal species based on the key from Quintero (1981) and Weygoldt (2002). The three studied species use the same habitats. During the day they can be found under rocks, in caves, in large trees with buttresses, and in burrows, where they hide and predate on arthropods, and during the night can be found on rocks and large trees from the floor to 2 m high (Dias & Machado, 2006; Weygoldt, 2000). In French Guiana, to limit our impact on population dynamics in this area known for harboring low density, the terminal parts (1 to 1.5 cm) of the antenniform leg tarsus were removed from live captured individuals before releasing the specimens at the same site, with a complete specimen of each species conserved in the collections at the University of Burgundy. The leg part, which was sufficient for DNA analysis, was situated after the autotomy point on the leg and can regenerate after molting (Weygoldt, 2000). As individuals were released at the same site of capture, they were marked by a small spot of Tipp-Ex® (type correction fluid) on the cuticle before release to avoid sampling them twice. This mark resists the high

hygrometry of the habitats, does not injure the animals, and is removed after molting. The samples were stored on site in 96% ethanol.

In Brazil, we used entire specimens conserved in the collections of the Universidad Federal do Piaui and the Universidade Federal do Mato Grosso. The specimens were stored in 96% ethanol. The terminal part of an antenniform leg (1 cm) was used for DNA analysis.

Total genomic DNA was extracted following a phenol-chloroform protocol modified from Hillis et al. (1996) using three to six apical segments of an antenniform leg tarsus. Polymerase chain reaction (PCR) DNA amplification targeting the mitochondrial-encoded *COI* gene was performed using a final volume of 30 µL of 1X buffer (Eppendorf Inc. France), including ca 5 ng genomic DNA, 0.15 U Hotstart DNA polymerase (Eppendorf Inc. France), 200 µmol/L dNTPs, and 200 nmol/L forward LCO1490 and reverse HCO2198 primers (Folmer et al., 1994). PCR cycling parameters were as follows: initial denaturation for 3 min at 95 °C, followed by 35 cycles of 20 s at 95 °C, 45 s at 40 °C, and 1 min at 65 °C, terminating with a final elongation at 65 °C for 2 min. PCR amplifications were performed with a Dyal DNA Engine BioRad thermocycler (Biorad, France). A 2 µL aliquot of each PCR product was checked to ensure it was the expected size (ca 710 bp) prior to purification with ExoSAP-IT Express (Thermo Fisher Scientific, France) according to the manufacturer's recommendations. Purified PCR products were sent to Macrogen Europe for sequencing using a BigDye Terminator 3.1 Sequencing kit and electrophoresed using an ABI 3730XL Genetic Analyzer (Applied Biosystems Inc.,

USA).

Newly produced sequences were edited with Genious R10.2.2 (Kearse et al., 2012) and all 63 sequences were deposited in GenBank (accession Nos.: MT899086–MT899148) and BOLD SYSTEMS (project AMBLY) (Ratnasingham & Hebert, 2007). All sequences (including two sequences already published by Arabi et al. (2012) *H. alces* JN018118 and *H. longicornis* JN018119) were aligned using the MAFFT V7 algorithm (Katoh & Standley, 2013) and trimmed to a final dataset of 570 bp sequences for Automatic Barcode Gap Discovery (ABGD), Bayesian implementation of Poisson Tree Processes (bPTP), and maximum-likelihood (ML) analyses (see below).

To explore the number of MOTUs that could represent putative cryptic species within the morphospecies, we applied three different approaches, two based on genetic distance, i.e., ABGD (Puillandre et al., 2012) and Barcode Index Number (BIN) (Ratnasingham & Hebert, 2013), and one based on phylogenetic tree analysis using bPTP (Zhang et al., 2013).

Both BIN and ABGD share the principle of clustering sequences into MOTUs according to their molecular divergence using algorithms to find discontinuities, i.e., barcoding gaps, between clusters. For the ABGD method, we used primary partitions as a basis for group definition, *P*-distance model, and the default value of 0.001 the minimum intraspecific distance. The BIN algorithm is implemented in the Barcode of Life Data system (BOLD; Ratnasingham & Hebert, 2007). Sequences deposited in BOLD or mined by BOLD from GenBank are clustered and each cluster is ascribed a

globally unique and specific identifier (aka BIN). In addition, BINs may be registered and made publicly available through BOLD, allowing tractability and have similar properties as classic taxonomy descriptors (e.g., can be split or synonymized).

The tree-based bPTP method assumes the branch lengths of a non-ultrametric phylogenetic tree to be generated by two independent classes of Poisson events, one corresponding to speciation and one to coalescence (Zhang et al., 2013). Additionally, the Bayesian version of the PTP method adds Bayesian support values (BS) for the delimited "species" (Zhang et al., 2013). For the input tree, we used an ML tree obtained using Mega-X (Kumar et al., 2018) from the haplotype data. The ML analysis was run under a thorough tree search with the HKY+G+I model of substitution i.e., Hasegawa-Kishino-Yano model (HKY, Hasegawa et al., 1985), with a discrete Gamma rate difference among sites (+G) and some sites being evolutionarily invariable (+I). Scorpionidae *Pandinus cavimanus* (GenBank accession number AY156580) was used as an outgroup. The bPTP analysis was performed on the bPTP web server (available at: http://www.species.h-its.org/ptp/) with 250 000 MCMC iterations, 10% burn-in, and outgroup exclusion.

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