A NEW SPECIES OF SOLAROPSIS BECK, 1837 (GASTROPODA: STYLOMMATOPHORA: SOLAROPSIDAE) FROM THE BRAZILIAN AMAZON

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ABSTRACT: A specimen of Solaropsidae from the collection of the Academy of Natural Sciences of Drexel University (Philadelphia, USA) was recognised as a potential new species based on shell morphology. With support from a multi-locus molecular phylogenetic analysis, a new species is described here: Solaropsis penthesileae sp. nov. It is native to the Amazon Rainforest in Pará state, northern Brazil, and it is closely related to S. nimbus (Simone). It differs in its more discoid shell, with a wider body whorl that bears a stronger median angulation in its lower spire. The specimen was collected in 1998 and is an example of the long shelf-life invertebrates may have in museum collections before they are identified and formally described. It is also a reminder of the importance of those collections for biodiversity studies.

KEY WORDS: Amazon; land snails; Pará; Solaropsidae; Solaropsis penthesileae sp. nov., sundial snails

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INTRODUCTION

The discovery of new species mostly happens not in the field, but rather in museum collection stores (ALLMON 1994, KEMP 2015). The material collected during expeditions, surveys, or by chance, will often remain undisturbed in museum cabinets for decades, awaiting discovery. As such, museums are a source of potential new species (GREEN 1998). There is an average of 21 years of the so-called “shelf-life” separating the time when a specimen is first collected from the formal description and naming of a new species in a scientific publication (FONTAINE et al. 2012). However, the shelf-life can sometimes extend to over a century (SALVADOR & CAVALCARI 2014, KEMP 2015).

Fortuitously, while conducting research on Neotropical Stylomatophora, I came across a unique specimen in the collection of the Academy of Natural Sciences of Drexel University (Philadelphia, USA) belonging to the family Solaropsidae, the sundial snails. The specimen was remarkably similar to Solaropsis nimbus (Simone, 2010), described from the northernmost region of the Brazilian Amazon Rainforest and of a very unique morphology within the Solaropsidae (SIMONE 2010, CALCUTT et al. 2020). Nevertheless, that specimen was immediately diagnosable from S. nimbus based on shell morphology, raising the possibility that it represented a still undescribed species. Herein, a combination of morphological characters and genetic data is used to support the description of a new species of the Solaropsidae.
MATERIAL AND METHODS

The specimen studied here belongs to the collection of the Academy of Natural Sciences of Drexel University (Philadelphia, USA), registration number ANSP A473903. It was fixed in ethanol and preserved in ethanol 80%. The snail was collected in the Floresta Nacional de Caxiuanã (“Caxiuanã National Forest”; see discussion below), which is a reserve in the lower Amazon. To my knowledge, no survey of the terrestrial mollusc fauna of this important forest reserve has been published to date.

A small fraction of tissue from the specimen’s foot was clipped for DNA extraction using the QIAGEN DNAEasy® Blood & Tissue Kit (standard protocol). The choice of markers for sequencing was based on

Table 1. Species used in the present phylogenetic analysis, with GenBank registration numbers, locality data, and references to the original publications

| Species                        | CO1     | 16S     | 28S     | Provenance                                      | References                     |
|--------------------------------|---------|---------|---------|------------------------------------------------|---------------------------------|
| Discidae                       |         |         |         |                                                 |                                 |
| Discus rotundatus (O.F. Müller, 1774) | FJ917285 | FJ917265 | FJ917240 | Germany: Hesse, Frankfurt am Main               | DINAPOLI & KLUSSMANN-KOLB 2010 |
| Sagidae                        |         |         |         |                                                 |                                 |
| Polyodontes acutangula (Burrow, 1815) | –       | KF246985 | KF207686 | Puerto Rico: Rio Grande                         | ROSENBERG et al. unpublished    |
| Platyssicinae portoricensis (Shuttleworth, 1854) | –       | KF247011 | KF207714 | Puerto Rico: Ponce                               | SEI et al. 2017                 |
| Sagda cf. epistylioides (Férussac, 1821) | –       | KF247005 | KF207708 | Jamaica: Westmoreland Parish                     | SEI et al. 2017                 |
| Solaropsidae                   |         |         |         |                                                 |                                 |
| Caracolus caracollus (Linnaeus, 1758) | –       | KF246970 | KF207671 | Puerto Rico: Jayuya                              | SEI et al. 2017                 |
| Caracolus sagemon (Beck, 1837)  | –       | KF246973 | KF207674 | Cuba: Holguin                                    | SEI et al. 2017                 |
| Solaropsis cf. gibboni (L. Pfeiffer,1846) | –       | KF246988 | KF207688 | Colombia: Cundinamarca                           | SEI et al. 2017                 |
| Solaropsis cf. juruana (Ihering, 1905) | MT080614 | MT080821 | MT080838 | Brazil: Pará, Altamira, Usina de Belo Monte     | CALCUTT et al. 2020             |
| Solaropsis heliaca (d’Orbigny,1835) | –       | KF246989 | KF207689 | Bolivia: Santa Cruz                              | SEI et al. 2017                 |
| Solaropsis juruana (Ihering, 1905) | MT080616 | MT080813 | MT080840 | Brazil: Acre, Rio Branco, Bairro Universitário   | CALCUTT et al. 2020             |
| Solaropsis nimbus (Simone, 2010) | MT080613 | MT080826 | MT080837 | Brazil: Amazonas, Pico da Nebína                 | CALCUTT et al. 2020             |
| Solaropsis penthesileae sp. nov. | MZ313463 | –       | MZ313211 | Brazil: Pará, Caxiuanã                           | Present work                    |
| Solaropsis punctata (Wagner, 1827) | MT080619 | MT080824 | –       | Brazil: Bahia, Parque Nacional Monte Pascoal     | CALCUTT et al. 2020             |
| Solaropsis rosaria (Pfeiffer, 1850) | MT080617 | MT080825 | MT080841 | Brazil: Goiás, Parque Estadual da Terra Ronca    | CALCUTT et al. 2020             |
| Solaropsis rugifera (Dhorn, 1882) | MT080618 | MT080822 | MT080842 | Brazil: Roraima, Porto Velho                      | CALCUTT et al. 2020             |
| Solaropsis sp. 1                | –       | KF246990 | KF207690 | Ecuador: El Zarza                                 | SEI et al. 2017                 |
| Solaropsis sp. 2                | –       | KF246991 | KF207691 | Paraguay: Concepción                             | SEI et al. 2017                 |
| Zachrysiidae                    |         |         |         |                                                 |                                 |
| Zachrysea havanensis (Pilsbry, 1894) | –       | KF246992 | KF207692 | Puerto Rico: Mayague                            | SEI et al. 2017                 |
the previous Solaropsidae phylogeny of Calcutt et al. (2020): (1) the barcoding fragment of the mitochondrial COI gene (primers LCO/HCO; Folmer et al. 1994); (2) the mitochondrial 16S rRNA gene (primers 16SarL and 16SbrH; Simon et al. 1994); (3) the 5’ end of the 28S rRNA gene (primers LSU-1/LSU-3 and LSU-2/LSU-5; Wade & Mordan 2000, Wade et al. 2006). PCR settings were as follows: (1) COI: initial denaturation at 96 °C (3 min); 35 cycles of denaturation at 95 °C (30 s), annealing at 48 °C (1 min), and extension at 72 °C (2 min); final extension at 72 °C (5 min); (2) 16S: same as COI, but 46 °C annealing temperature; (3) 28S: initial denaturation at 95 °C (3 min); 40 cycles of denaturation at 95 °C (30 s), annealing at either 50 °C (LSU-1/LSU-3 fragment) or 45 °C (LSU-2/LSU-5 fragment) (1 min), and (4) extension at 72 °C (5 min for LSU-1/LSU-3 fragment or 2 min for LSU-2/LSU-5 fragment); final extension at 72 °C (4 min). The PCR products were then quantified via agarose gel electrophoresis, followed by cleaning with ExoSAP-IT™ (Affymetrix Inc.), and sent out for Sanger sequencing. The sequences were assembled in Geneious Prime (v. 2020.2.2, Biomatters Ltd.), quality-proofed, and uploaded to NCBI GenBank.

Phylogenetic analysis was conducted to define the position of the specimen within the Solaropsidae, using all available sequences of this family (Table 1) from the studies of Sei et al. (2017) and Calcutt et al. (2020). Other taxa of the Sagdoidea and a taxon of the Discoidea were chosen as outgroups (Table 1). Alignment of sequences was conducted in Geneious Prime through the MUSCLE plugin (Edgar 2004) with default settings (i.e. optimised for accuracy). The alignment of each marker was manually proofed for errors in Geneious and then run through Gblocks (Talavera & Castresana 2007; using the least restrictive settings) to eliminate poorly-aligned and divergent positions that could interfere with the phylogenetic analysis.

The phylogenetic analysis (Bayesian inference) was performed with the MrBayes (v.3.2.7; Ronquist et al. 2012) plugin in Geneious. Two concurrent analyses were run, each with four Markov chains of 15 million generations (the first 20% discarded as ‘burn-in’), the default priors, nst = 6, rates = invgamma, temperature parameter = 0.1, sampling every 1,000 generations; substitution model parameters were unlinked across the three markers. Markov chain Monte Carlo (MCMC) convergence was assessed by examining the standard deviation of split frequencies (<0.01) and trace plots in Geneious.

RESULTS

The COI and 28S markers of the ANSP specimen were successfully sequenced (GenBank accession numbers MZ313211 and MZ313463, respectively), but the 16S marker could not be successfully amplified after several attempts and variations in the PCR protocol. The 28S fragment amplifies together with the ITS2 marker, which is present in the GenBank record though it was not used for the phylogenetic analysis.

After running the sequence alignment of each marker through Gblocks, 571 informative positions were retained for the COI marker (out of 638), 438 were retained for 16S (out of ca. 528), and 423 were retained for 28S (out of 448).

The resulting Bayesian tree (Fig. 1) showed strong support for most of the solaropsid clades, despite the polytomy. The position of the Caracolinae and the family rank of the Zachrysiidae remains ambiguous, as already noted by Calcutt et al. 2020. More importantly, the present ANSP specimen has perfect support (posterior probability = 1) as the sister taxon of S. nimbus, as predicted by its morphology. Those two terminals are more genetically distant from one another than other well-supported crown Solaropsis (Fig. 1). In particular, the COI sequences of the ANSP specimen and that of S. nimbus have between 91.2% and 93.7% identity, depending on the reading of some ambiguous bases. These results support the suspicion that the ANSP specimen represents a new species.
SYSTEMATICS

Superfamily Sagdoidea Pilsbr, 1895
Family Solaropsidae H. Nordsieck, 1986
Genus Solaropsis H. Beck, 1837

Type species: Helix pellisserpentis Gmelin, 1791, by subsequent designation; junior synonym of Solaropsis undata ([Lightfoot], 1786).

Solaropsis penthesileae sp. nov.
Figs 2–6
LSID D4F10593-C526-4099-9486-B2C9C6630EA4

Type locality. Brazil, Pará state, Caxiuanã, Fazenda. Holotype. ANSP A473903 (R. Newman leg., 17/VI/1998; ex Delaware Museum of Natural History collection).

Description. Shell (Fig. 2) small, globular to discoid. Protoconch (Fig. 5) smooth, 1 1/2 whorl, of uniform light ochre colour; transition to teleoconch marked by faint callus and onset of colour pattern. Shell colour ochre, with patterns in darker shades of brown, consisting of square markings on shoulder (Fig. 3) and three spiral dotted lines (one on the shoulder, one immediately above the median angulation, and one on the base of the whorl). Periostracum almost uniformly covered by small hairs (Fig. 6), except for abapical area of shell. Teleoconch smooth, except for very faint growth lines, but hairs give it a dotted appearance. Suture shallow. Whorls uniformly increasing in height and width (Figs 2, 3). Body whorl wide, with a faint sub-sutural shoulder and a weak median angulation (Fig. 2). Precise morphology of aperture and umbilicus cannot be assessed, due to the specimen not having the typical adult reflected peristome (Fig. 4). Aperture apparently D-shaped, small, abapically positioned in relation to body whorl (Fig. 4).

Measurements. Holotype: shell height = 10.8 mm; shell width = 15.3 mm.
Sequence data. GenBank accession numbers MZ313463 (COI) and MZ313211 (ITS2+28S).

Distribution. Known only from the type locality, Caxiuanã, which refers to the Floresta Nacional de Caxiuanã (“Caxiuanã National Forest”), in Melgaço municipality. The term used for the precise locality was “Fazenda” [=farm], which potentially means the research station in the forest (Ferreira Penna Scientific Station, coordinates: 01°47'32.3"S, 51°26'02.5"W).

Habitat. The specimen was found on an açaí palm (*Euterpe oleracea* Mart.), but there is not enough information to assess whether it was in a forested area or an anthropically-disturbed one. In any event, the Caxiuanã Forest is part of the Amazon Rainforest biome and is notable by its ‘terra firma’ forests, which do not flood seasonally and make up around 85% of Caxiuanã’s total area (Lisboa 1997). A smaller fraction (ca. 12%) of the Caxiuanã includes floodplain forests as well (Lisboa 1997).

Etymology. The new species is named after Penthesilea, the Amazonian queen from Greek mythology. Suggested vernacular name: Penthesilea’s sundial.

Remarks. Conchologically, *S. penthesileae* sp. nov. (Figs 2–6) is most similar to *S. nimbus* (Figs 7–8) from the neighbouring Amazonas state. This is also shown by their sister-taxon relationship in the phylogeny (Fig. 1) and the pairwise identity between their COI sequences. *S. penthesileae* sp. nov. can be diagnosed by its more discoid shell, with a wider body whorl that bears a stronger median angulation, and a lower spire. The adult shell of *S. penthesileae* sp. nov. would supposedly be wider, but only one specimen of the species and two specimens of *S. nimbus* are known (Simone 2010), precluding any meaningful comparison in that regard.

A further similar species is *S. aff. rosaria* (L. Pfeiffer, 1850), reported from Tocantins and Minas Gerais states (Salvador et al. 2015, 2021). However, *S. penthesileae* sp. nov. is much smaller, with a more delicate general appearance, a more prominently raised spire, stronger median angulation on the body whorl, and a smaller and more circular aperture, positioned more abapically in relation to body whorl. *S. juruana* Ihering, 1905, known from Acre state and western Amazonas state, has a similar colour pattern, but is slightly bigger and has a fully discoid shell. Both *S. rosaria* and *S. juruana* are placed within the crown group in the present phylogeny (Fig. 1).

Figs 2–6. *Solaropsis penthesileae* sp. nov., holotype ANSP A473903 (shell height = 10.8 mm; shell width = 15.3 mm): 5 – detail of protoconch; 6 – detail of teleoconch showing the hairs. Scale bars 5 mm (2–4, 6) and 1 mm (5)
**DISCUSSION**

*S. penthesileae* sp. nov. was found in a region of Brazil which has been malacologically neglected (SALVADOR 2019). Its closest relative, *S. nimbus*, is known from Pico da Neblina (“Neblina Peak”), in Amazonas state, which is located ca. 1,600 km west-northwest of Caxiuanã Forest. The Amazon is the most biodiverse biome in the world, but its invertebrates, and molluscs in particular, are still poorly understood (MITTERMEIER et al. 2003, LIMA et al. 2021). Only about 15% of the land snail species known to occur in Brazil have been recorded from the Amazon (SALVADOR 2019).

Given the undersampling of the Amazon and its gargantuan area, it is to be expected that many species are yet to come to light. It is thus not surprising that some of those new species are already part of museum collections, just waiting to be studied. The specimen studied here was collected in 1998 and is an example of the long shelf-life invertebrates may have in museum collections before they are identified and formally described. It is also a reminder of the importance of those collections for biodiversity studies (ALLMON 1994).

Unfortunately, Brazilian environmental policies have taken a turn for the worse during the catastrophic current administration (e.g. TOLLEFSON 2019). Thus, in all likelihood many new invertebrate species are being lost in the Amazon before being recognised and described, in a similar manner to what has been observed in other tropical areas (e.g. RICHLING & BOUCHET 2013, SIMONE & SALVADOR 2016).

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