## 2021 S.T. Yau High School Science Award

## Research Report

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### Title of Research Report

A molecular phylogeny of cavernicolous Oniscidea (Isopoda) in Southern China reveals multiple origins of troglodytic behavior and a new species of blind Armadillidae (Oniscidea, Isopoda)

### Date

[2021/09/12]

# A molecular phylogeny of cavernicolous Oniscidea (Isopoda) in Southern China reveals multiple origins of troglodytic behavior and a new species of blind Armadillidae (Oniscidea, Isopoda)

Bob Guan

## Abstract

Despite the high diversity of Oniscidea in the Guangxi province, with many rock-face dwelling and fully troglobitic species, we still lack a comprehensive phylogeny for them. We infer these relationships in this paper by utilizing the genetic markers COI and 16S and build a topology using the Maximum Likelihood and Bayesian Inference methods. By comparing the phylogeny of Guangxi Oniscidea with that of other related taxa, we found troglobitic behavior to have arisen multiple times through convergent evolution, and the genera *Spherillo* and *Burmoniscus* to be in need of revision. Additionally, we discovered a new eyeless and pigment-lacking species by using morphology and molecular biology in conjunction.

**Keywords**: genetic markers, convergent evolution, monophyly, taxonomic revision, caves, southern China, new species, woodlice, Oniscidea.

# Declaration of Academic Integrity

The participating team declares that the paper submitted is comprised of original research and results obtained under the guidance of the instructor. To the team's best knowledge, the paper does not contain research results, published or not, from a person who is not a team member, except for the content listed in the references and the acknowledgment. If there is any misinformation, we are willing to take all the related responsibilities.

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2021/9/2

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# I. INTRODUCTION

## *A. **A global overview of Oniscidea***

Oniscidea[1] is a polyphyletic (Lins 2017) suborder within the Order Isopoda (Superorder Peracarida, Subclass Eumalacostraca, Class Malacostraca, Subphylum Crustacea, Phylum Arthropoda, Kingdom Animalia) and contributes to 50% of its species (Martin 2001). As of 2014, 3710 species belonging to 527 genera and 37 families within Oniscidea have been validated in literature, with total number being estimated to be between 5000 and 7000. Of these 37 families, the three with the highest species diversity in descending order are Armadillidae[2], Philosciidae[3], and Trichoniscidae[4] (Sfendourakis 2015).

Most Oniscidea species inhabit terrestrial habitats and serve as keystone detritivores because of their high population density and phytosaprophagy[5] (Dias and Hassall 2005), and have penetrated every biome of the world, apart from the poles and altitudes of above 4800 m (Beron 1997). Despite their high diversity, they are relatively poorly adapted to land, with rapid water loss to environment occurring in air that is not saturated (Spencer 1954). The families that are best adapted to terrestrial environments, such as Oniscidae, Porcellionidae, and Armadillidiidae, all possess morphological specialization in the form of invaginated exopodite lungs to prevent desiccation (Wright and Ting 2006). Certain species, such as *Armadillidium vulgare*[6] (Latreille 1804), are able to conglobate. They use this to reduce transpiration and as a defense mechanism against predation (Smigel and Gibbs 2008).

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[1] Known commonly as woodlice, all binomial names to appear henceforth belongs to this group unless specified

[2] A common group of woodlice which are able to curve into a sphere

[3] A common group of woodlice which are intolerant to dry conditions

[4] A common group of woodlice with advanced gas exchange surfaces

[5] Decomposition of dead plant matter

[6] Known by the common name of common pill-bug

### *B.* ***The morphological characteristics of Oniscidea***

Oniscidea isopods are typically dorsoventrally[7] flattened, though the flattening is not striking when it comes to certain families such as Armadillidiidae. Like other isopods, their bodies are conspicuously segmented. The main body covering of Oniscidea is the cuticle, which is molted periodically. These cuticles form sclerites through the deposition of calcium salts. Interscerital cuticles are soft and flexible to allow motion. These areas are known as articular membranes (Shi 2012).

An Oniscidea body consists of an anterior head, a middle thorax, and a posterior abdomen. The head consists of 5 segments, the thorax consists of 8, and the abdomen consists of 6. Thoratic segments are known as thoracomeres, and abdominal ones are known as pleomeres. These ancestral tagmata are modified by the inclusion of one thoracomere with the head to form a cephalothorax. The remaining 7 thoracomeres form the pereon, and are referred as pereomeres. The segments of all three tagmata bear appendages.

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[7] From top to bottom

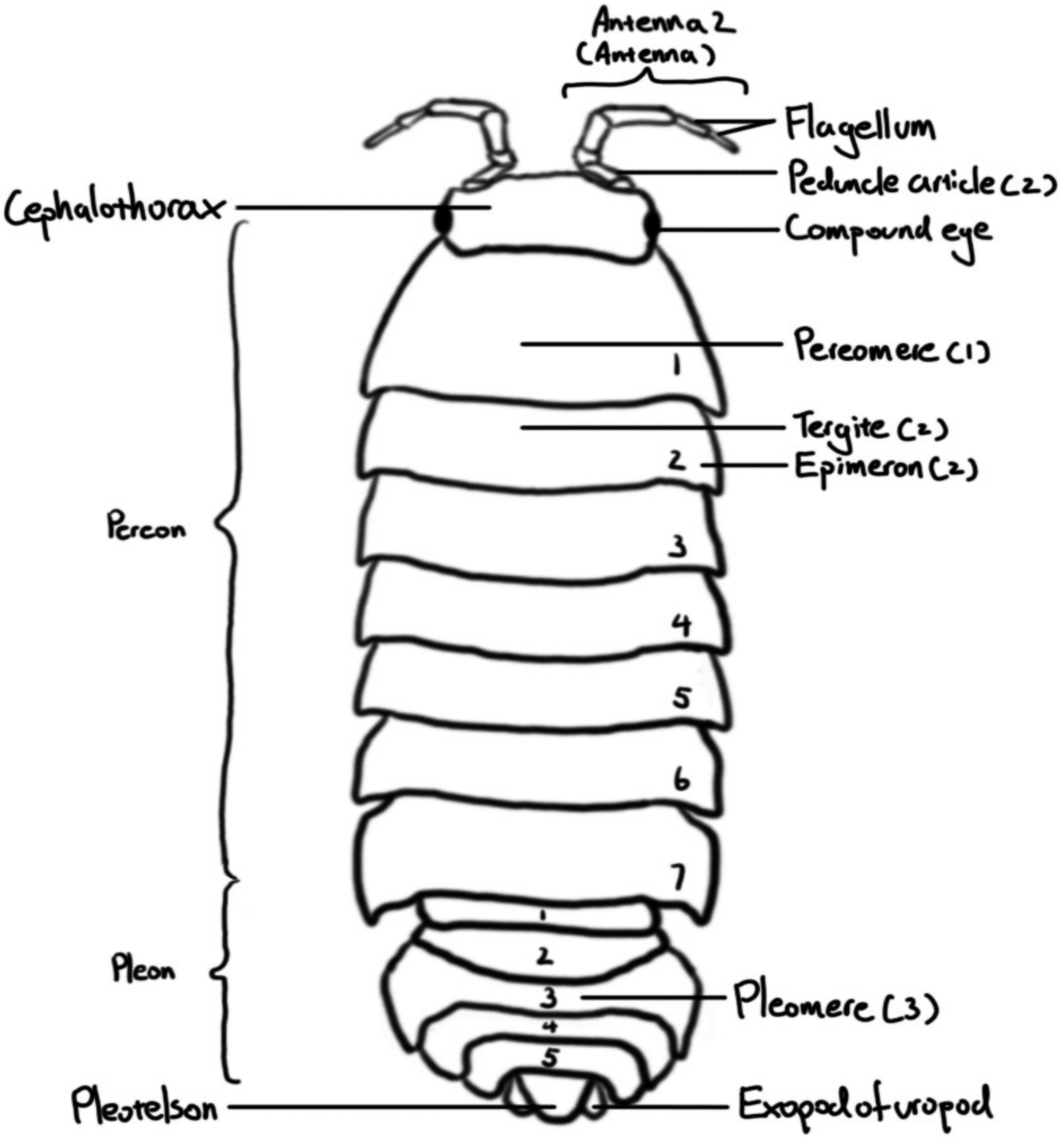

Fig 1. A labeled dorsal view of an Armadillididae isopod; segment number is presented in brackets, drawn by Bob Guan.

The small cephalothorax is visibly the first tagma[8]. It bears eyes, 5 pairs of head appendages, and a pair of chewing maxillipeds. The separate segments making it up are fused and indistinguishable. Oniscidea isopods have two pairs of antennae. The first pair is extremely reduced, and contains three segments. These likely serve as olfactory organs. The second pair is hypertrophied, and used as sensory organs. They contain 7 segments overall, with 5 in the peduncle close to the body, and 2 in the flagellum away from the body. The mouthparts of an Oniscidea isopod are asymmetric, with a convex part and a concave part. These mouthparts are covered and hidden by the maxillipeds.

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[8] Grouping of multiple segments

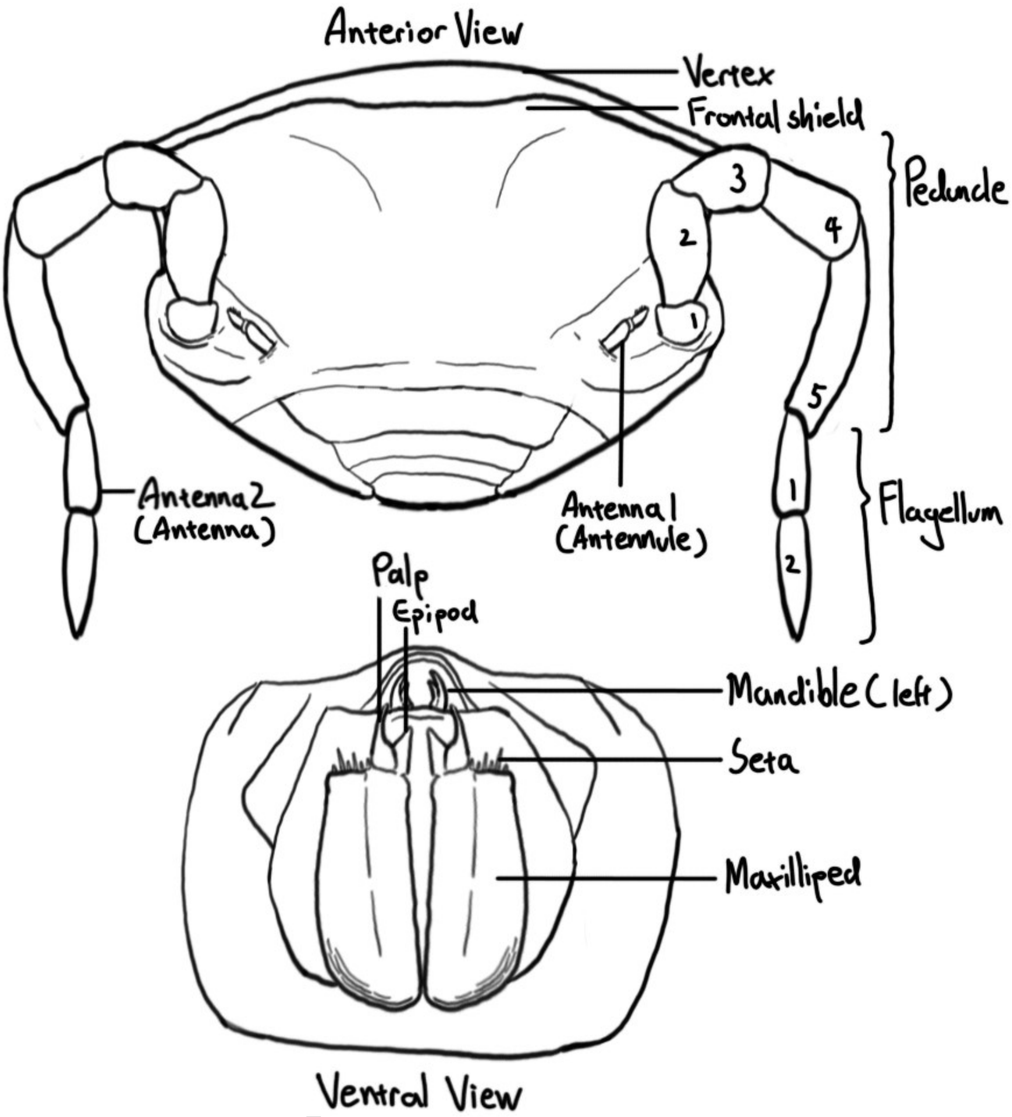

Fig 2. Labeled anterior and ventral views of the head of an Armadillididae isopod, drawn by Bob Guan.

The cephalothorax fits into a median notch on the anterior of the first pereomere. The pereomeres are covered by an exoskeletal ring composed of 2 sclerites. In reproductively active females the sternites are hidden by thin membranous oostegites. Oostegite are soft and pliable, and form the marsupium, used to carry eggs and the first few stages of mancaelarva. The lateral extremities of the tergites reach ventrally far below the sternites. These extensions are the epimera, also known as side plates. These plates are pronounced in several species of Oniscidea, such as *Cubaris murina* and *Porcellio weneri*. The 7 pairs of pereopods resemble each other and each consist of 7 articles. From order of closest to the body to furthest from the body, these articles are called the coxa, basis, ischium, merus, carpus, propodus, and dactyl, respectively. The male gonopods are on the 7th pereomere as a genital papilla, which is a transparent membranous triangle overlapping the bases of the anterior abdominal appendages. The female gonopores are on pereomere 5, and are hard to see.

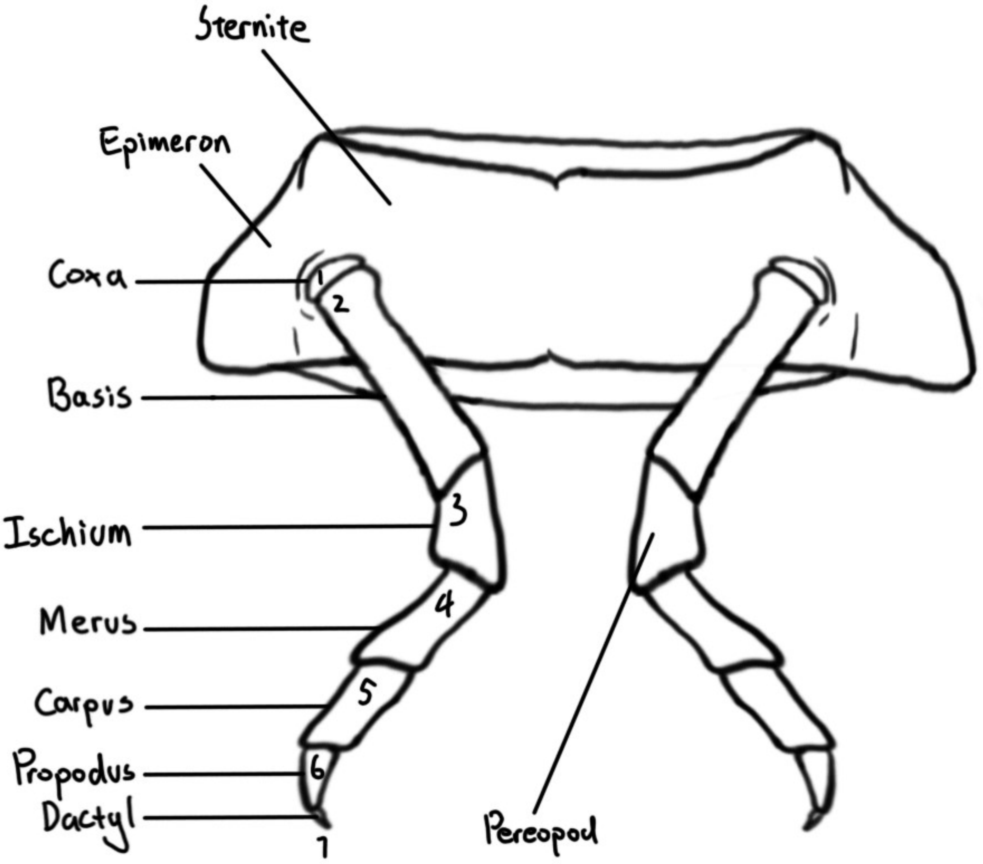

Fig 3. Labeled pereomere and pereopods of an Armadillididae isopod, drawn by Bob Guan.

The abdomen is composed of 6 segments, the last one is fused with the telson to form a pleotelson, while the first 5 are independent and together form the pleon. Pleon segments are called pleomeres, and their appendages pleopods. The appendages of the 6th segment are called uropods. The biramous[9] pleopods consist of a basal protopods and 2 rami. The medial rami, also known as endopods, are soft with thin cuticles, and are the gas exchange surfaces. They are covered and protected by the lateral rami, known as exopods. The long slender endopods of the first 2 pleopods in males are also gonopods, used to deliver sperm. The anterior exopods of the pleopods contain pseudotraches known as the corpus alatum to supplement the gills. In living specimens, they are easily seen as large white masses. The uropods are the appendages of the 6th abdominal segment. They are biramous and heavily sclerotized. In most species the

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[9] Dividing to form two branches

rami are long and dorsally visible, but in some genera, such as *Armadillidium*, the rami are short, so they can be protected when the animal conglobates.

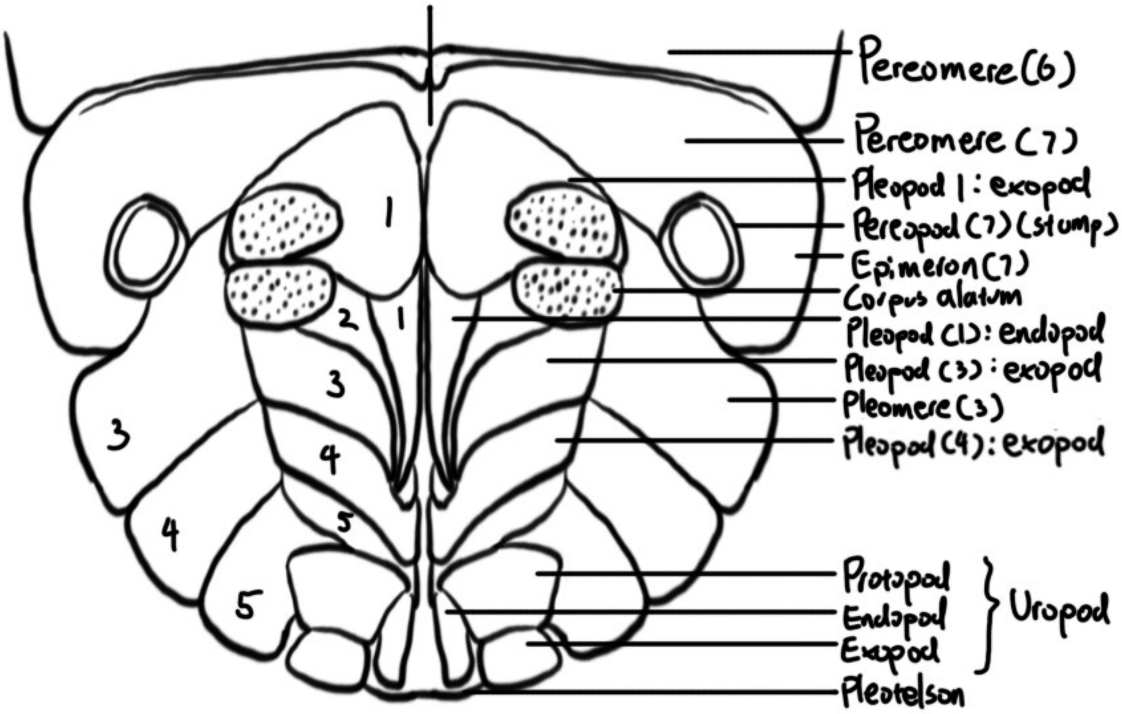

Fig 4. Labeled ventral view of the pleon of a male Armadillididae isopod; segment number is presented in brackets, drawn by Bob Guan

*C. **Current molecular studies of Oniscidea Phylogeny***

Despite the advancements in molecular phylogeny, generally considered to be the more accurate option as convergent evolution may cause distantly related clades to have similar characteristics, most modern-day studies of Oniscidea are still based on morphology. Molecular studies of Oniscidea are rare (Lins 2017, Dimitriou 2018), and studies that take both into account are nonexistent.

Though Oniscidea was presumed to be monophyletic in the past based on morphological characteristics (Schmidt 2008), molecular analyses have shed light on the likely polyphyly of both the suborder, and a family inside it (Lins 2017, Dimitriou 2018). Analyses of mito-nuclear genes suggests the families Tylidae and Ligiidae to be placed outside of the main Oniscidea group (Mesoniscidae, Crinocheta, and Synocheta), making it more likely that they colonized land independently (Lins 2017), while molecular analyses of the family Porcellionidae based on the CO*I*, *16S*, *18S*, *28S*, and *NAK* sequences reveals both it, and its most diverse genus, *Porcellio*[10], to be polyphyletic (Dimitriou 2018). Such large inconsistencies between molecular analysis and morphological analysis makes expanding current molecular studies a priority.

### *D. **The distribution and research of Oniscidea within China***

As of 2005, 72 species of terrestrial Oniscidea isopods have been described within China, of which 28% inhabit tropical regions, and 50% are unique to China. This apparent lack of diversity is because of the initial lack of interest within the Oniscidea field in China, with new species being reported more frequently now. In contrast to this, the real diversity of Chinese Oniscidea seems to be quite large, with strong zonal characteristics (Chen 2005).

Many Chinese studies of Oniscidea since the 1990s have focused on their ecological importance and morphological characteristics, with a lesser focus on their phylogeny (Liao 1992, Tang 2008, Chen 2003). Most studies of Oniscidea are restricted to south-eastern China, or other coastal areas, with especially many describing the species of the Yangtze river's downstream regions (Kwon 1996, Chen 1987, Gui 1994, Tang 1994). Though some studies of Oniscidea have been made in southern China, they are limited to the Yunnan province and the Hong Kong SAR (Dai and Cai 1998, Kwon 1993), and do not describe phylogeny.

Despite the considerable diversity of cavernicolous Onscidea isopods within the Guangxi province (Taiti and Gruber 2008, Taiti and Xue 2012), as well as the establishment of phylogenic studies of non-Chinese cavernicolous Oniscidea isopods (Campos-Filho 2020), no studies so far have attempted to resolve the phylogeny between them, rock-face Oniscidea, and non-cavernicolous Oniscidea, as well as their position in the phylogenetic tree of Oniscidea as a whole. In addition to this, no studies have been made so far using phylogeny to describe Chinese cavernicolous Oniscidea. This paper aims to provide all three using molecular biology.

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[10] A genus which includes the common rough isopod, *Porcellio scaber* Latreille, 1804

# II. MATERIALS AND METHODS

## *A.* ***Sampling***

Oniscidea specimens were collected in the Guangxi province in several regions. Three species were collected in the Detian International Waterfall Park (North 22 degrees 51’22’’, East 106 degrees 43’28’’, Altitude 450 meters, 2021/7/6) at the Sino-Vietnamese border near Chongzuo. Two species were procured in Nanning from a vendor which collected them in the Longzhou prefecture in Chongzuo (North 22 degrees 64’88’’, East 106 degrees 89’57’’, Altitude 158 meters, 2021/7/4). One species was collected in Beihai. Fresh specimens were kept alive initially and provided substrate of their region of collection, and placed in 99% alcohol when shipped. Genes of numerous other species from other regions of various different genera were also used in the phylogenetic analysis, as geography does not seem to affect phylogenetic relationships (Dimitriou 2018). Some of these genes were found in the methods of other papers (Dimitriou 2018, Lins 2017), while others were found using on the GenBank database. Genes of both sources were downloaded from the GenBank database.

Table 1. Species which were collected, locality of origin, and available sequence data for them.

| Species | Locality | Genes | |
|---|---|---|---|
| | | COI | 16s |
| *Spherillo sinoculus* n. sp. | Guangxi (Chongzuo) | √ | √ |
| *Burmoniscus* sp. 1 | Guangxi (Chongzuo) | √ | |
| Ligiidae sp. | Guangxi (Detian) | √ | √ |
| *Burmoniscus* sp. 2 | Guangxi (Detian) | √ | |
| *Spherillo* sp. | Guangxi (Detian) | | √ |
| *Porcellionides pruinosus* (Brandt, 1833) | Guangxi (Beihai) | √ | |

Table 2. Species which were accessed through papers, locality of origin, available and used sequence data for them, and access number

| Species (code) | Locality | Genes | | Access number |
|---|---|---|---|---|
| | | COI | 16s | |
| *Proporcellio vulcanius* (Verhoeff, 1908) | Cyprus (Larnaca) | √ | √ | MG887933/MG887948 |
| *Agabiformius excavatus* Verhoeff, 1941 | Cyprus (Paphos) | | √ | -/MG887955 |
| *A. excavates* | Cyprus (Paphos) | | √ | -/MG887956 |
| *Porcellio laevis* Latreille, 1804 | Cyprus (Lemesos) | √ | √ | MG887936/MG887957 |
| *P. laevis* | Cyprus (Lemesos) | √ | √ | MG887937/MG887958 |
| *P. pruinosus* | Cyprus (Larnaca) | √ | √ | MG887934/MG887949 |
| *P. pruinosus* | Cyprus (Larnaca) | √ | √ | MG887935/MG887950 |
| *Leptotrichus kosswigi* Strouhal, 1960 | Cyprus (Paphos) | | √ | -/MG887963 |
| *Porcellio nasutus* Strouhal, 1936 | Greece (Parnon) | √ | √ | MG887944/<br>MG887953 |
| *P. nasutus* | Greece (Parnon) | | √ | -/MG887954 |
| Tura sp. | Kenya (Mombasa) | √ | √ | MG887946/MG887966 |
| *Caeroplastes porphyrivagus* (Verhoeff, 1918) | France (Toulon) | √ | | MG887932/- |
| *Uramba triangulifera* Budde-Lund, 1910 | Kenya (Aberdare National Park ) | | √ | -/MG887961 |
| *Thermocellio* sp. | Tanzania (Dar es Salaam) | | √ | -/MG887962 |
| *Mica tardus* (BuddeLund, 1885) | Italy (Sardinia) | | √ | -/MG887959 |

| | | | | |
|---|---|---|---|---|
| *Acaeroplastes melanurus* (Budde-Lund, 1885) | Italy (Sardinia) | √ | √ | MG887945/G887960 |
| *Soteriscus laouensis* Taiti & Rossano, 2015 | Morocco (Tirinesse) | √ | √ | MG887931/MG887964 |
| *Trachelipus aegaeus* (Verhoeff, 1907) | Greece (Naxos) | √ | √ | EF659961/KF891440 |
| *Hemilepistus klugii* (Brandt, 1933 | Iran (Isfahan) | √ | √ | MG887938/MG887951 |
| *Hemilepistus schirazi* Lincoln, 1970 | Iran (Shahreza) | √ | √ | MG887939/MG887952 |
| *Armadillidium vulgare* (Latrteille, 1904) | Cyprus (Limassol) | √ | √ | KR424609/AJ419997 |
| *Chaetophiloscia elongata* (Dollfus, 1884) | Italy (Sardinia) | √ | √ | KJ668161/AJ388091 |
| *Actaecia euchroa* Dana, 1853) | New Zealand | √ | √ | GQ302701/AJ388093 |
| *Cubaris murina* Brandt, 1833 | n.a. | √ | | AB861536/- |
| *Armadillo officinalis* Dumeril, 1816 | n.a. | √ | | FN824109/- |
| *Spherillo dorsalis* Iwamoto, 1843 | n.a. | √ | | AB861899.1/- |
| *Trichoniscus pusillus* Brandt, 1833 | n.a. | √ | | FN824124.1/- |
| *Burmoniscus meeusei* (Holthuis, 1947) | n.a. | √ | | AB889796.1/- |
| *Oniscus asellus* Linnaeus 1758 | n.a. | √ | √ | KM611735/KX467635 |

|  |  |  |  |  |
|---|---|---|---|---|
| *Spherillo grossus* (Budde-Lund, 1885) | n.a. | √ | √ | KC706445/KC706402 |
| *Mongoloniscus sinensis* (Dollfus 1901) | n.a. | √ |  | KT424048/- |
| *Haloniscus* sp. 2 | n.a. | √ |  | KR424612/- |
| *Spherillo obscurus* (Budde-Lund, 1885) | n.a. |  | √ | -/AB861914 |
| *Merulana helmsiana* (Chilton 1916) | n.a. |  | √ | -/KC706379 |
| *Burmoniscus* sp. 3 | n.a. |  | √ | -/AB626480 |

### *B.* ***DNA extraction and amplification***

Specimens were used immediately upon arrival. Total genomic DNA was extracted from entire bodies using the AxyPrep Genomic DNA Miniprep Kit (AXYGEN), following the instructions of the manufacturer.

COI, 16S, 18S, and 28S rDNA were amplified using the following primer pairs respectively: COBU (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and COBL (3'-TAA ACT TCA GGG TG ACC AAA AAA TCA-5'), 16S-AR (5'-GCC GCA GTA THC TRA CTG TGC T-3') and 16S-BR (3'-CCG GTC TGA ACT CAG ATC ACG T-5'). All sequences were then put through PCR: pre-denaturation for 5 minutes at 94 C, 10 cycles of 30 seconds at 94 C, then 30 seconds at 48/54 C respectively, then 30 seconds at 72 C. This is then followed by 25 cycles of 30 seconds at 94 C, 30 seconds at 51 C, 30 seconds at 72 C, and then 7 minutes at 72 C. These products were then sent to a biotech company (BioSune, Shanghai, China) which sequenced them using sanger sequencing that corresponded to the primers.

### C. ***Phylogenetic analyses***

The wave function in ATGC was first used to manually correct and adjust nucleotides and to shorten strands. These amended strands for each sequence were then aligned with default parameters in MEGA7 (Kumar et al. 2016) through the muscle method, and manually cut into the same lengths by removing all parts where there were missing data. The sequences were then individually saved in the FASTA format, and put into PhyloSuite (Zhang et al. 2020). Within PhyloSuite, the flowchart function is used with the following parameters: sequence alignment with both MAFFT and MACSE, alignment cleaning with Gblocks, model selection with Concatenation and ModelFinder, Tree Reconstruction with IQ-TREE (Nguyen et al. 2014) and MrBayes (Ronquist et al. 2012). ModelFinder is preferred here over PartitionFinder, as even though it is slower, it is more accurate. Maximum likelihood analyses were made in IQ-TREE, and Bayesian inference analyses were made in MrBayes. This bilateral analysis with two models allows the final results to be compared to each other to check for inaccuracies.

### D. ***Equipment and procedure of dissection***

This paper adopts a simplified version of the morphological traits used to describe cavernicolous species in the Guangxi province (Taiti and Gruber 2008, Taiti and Xue 2012), as well as an Armadillidiidae oniscid (Hamaied 2018). The morphologies considered are as follows: a lateral view of the whole specimen, a dorsal view of the whole specimen, a dorsal view of the first pereopod, a ventral view of the first pereopod, a dorsal view of the seventh pereopod, a ventral view of the seventh pereopod, the antenna, the antennula, the endopods of the first pleopods, the exopods of the first pleopods, the maxillipeds, a ventral view of the cephalothorax, and a front view of the cephalothorax. A Leica dissection microscope was used to dissect all specimen.

# III. RESULTS

## A. ***Molecular***

46% (19/41) of the species had both targeted genes successfully amplified and sequenced, 24% (10/41) only yielded usable 16S results, and 29% (12/41) only yielded usable CO*I* results.

Maximum Likelihood and Bayesian Inference analyses resulted in two somewhat divergent, yet well supported topologies, with the Maximum Likelihood analysis being more well-supported, as well as more detailed. Both topologies broadly agree when it comes to the position of the possible new species, *Spherillo sinoculus*, as well as the phylogeny of its genus and its allies.

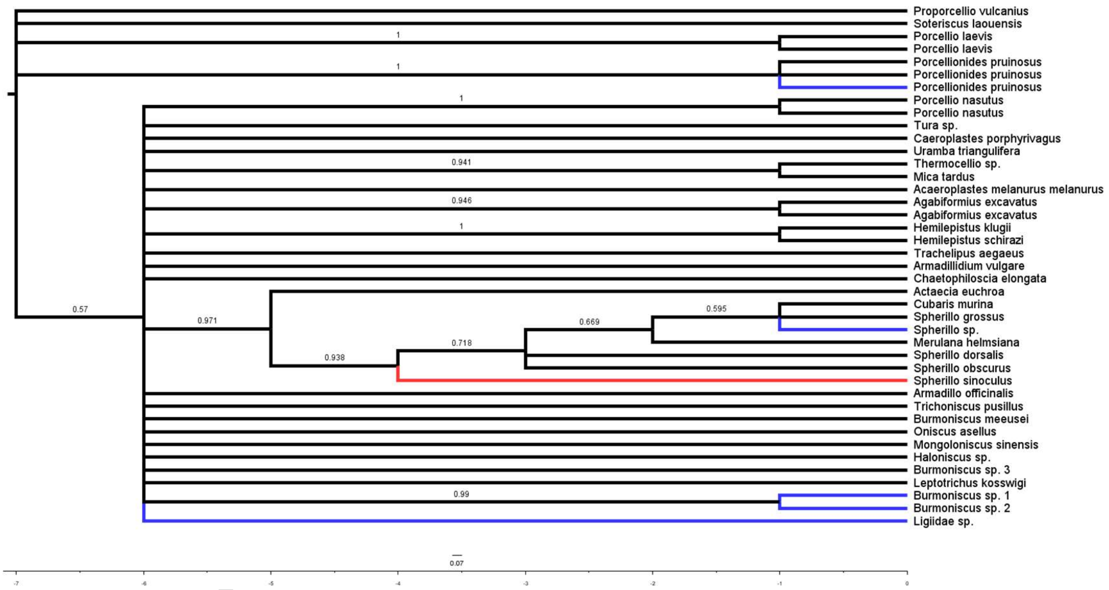

Fig. 5 Phylogram based on the genes COI and 16S, generated using Bayesian Analysis through the MrBayes algorithm. Statistic support is given on the branch preceding divergence. Specimen collected in Guangxi have their immediate branches colored blue, while the possible new species, *Spherillo sinoculus*, has its immediate branch colored red. Individuals of the same species are presented separately from each other, and not collapsed.

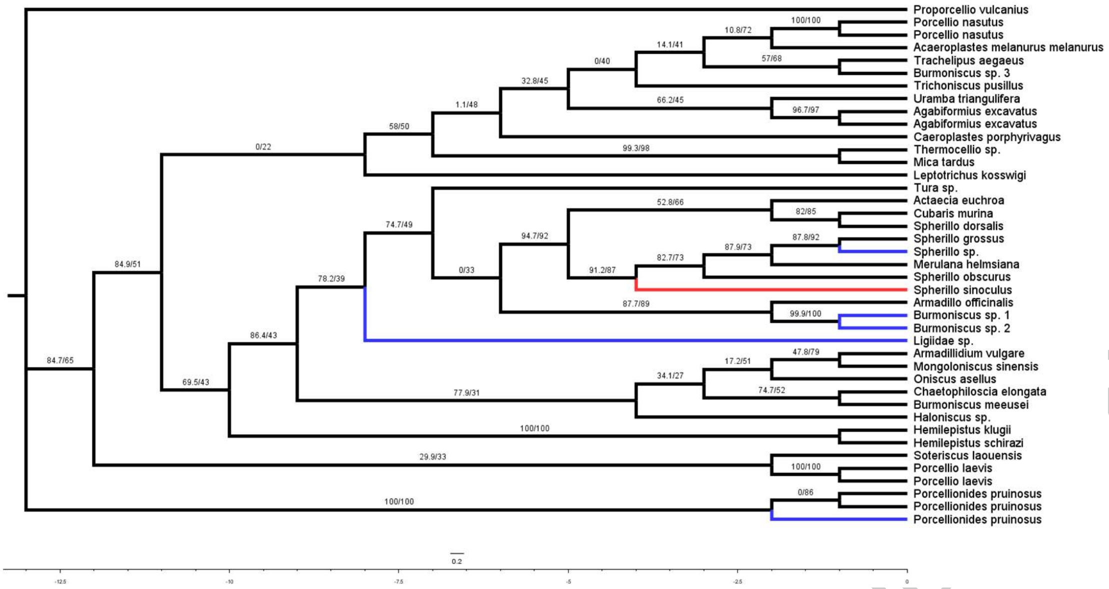

Fig. 6 Phylogram based on the genes COI and 16S, generated using Maximum Likelihood through the IQ-Tree algorithm. Statistic support is given on the branch preceding divergence. Specimen collected in Guangxi have their immediate branches colored blue, while the possible new species, *Spherillo sinoculus*, has its immediate branch colored red. Individuals of the same species are presented separately from each other, and not collapsed.

The monophyly of the genus *Spherillo* is put into doubt, as both Bayesian Inference and Maximum Likelihood show species of other genera (*Merulana helsiana* and *Cubaris murina*) to be more closely related to some *Spherillo* species (*Spherillo grossus*, *Spherillo dorsalis*, *Spherillo* sp.) than these species are to other *Spherillo* species.

*Spherillo sinoculus* is a sister group to most *Spherillo* species under both phylograms, placing it as the most basal *Spherillo* species. Because of the demonstrated polyphyly of the genus *Spherillo*, it is given this genus name as a placeholder, as it is the genus it is most closely related to, until more conclusive research can be done.

*Burmoniscus* sp. 1 and *Burmoniscus* sp. 2 are found to be closely related to each other, but not to *Burmoniscus meeusei* nor *Burmoniscus* sp. 3. *Burmoniscus* sp. 3 was also found to be distantly related from each other under both phylograms. The Bayesian Analysis results are inconclusive regarding the relationship between *Burmoniscus* sp. 1 and *Burmoniscus* sp. 2 with other species. The Maximum Likelihood results, on the other hand, are extremely well supported (87.7/89), and suggest them to be most closely related to *Armadillo officinalis*.

Ligiidae sp. Was found as a group parallel to all species belonging to the genera *Proporcellio, Soteriscus*, *Porcellio*, and *Porcellionides*, except for *Porcellio nasutus* based on Bayesian analysis. Based on Maximum Likelihood, it was found instead to be parallel to the group which includes all species from the genera *Tura*, *Actaecia*, *Cubaris*, *Spherillo*, *Merulana*, *Armadillo*, and *Burmoniscus*, apart from the species *Burmoniscus meeusei* as well as *Burmoniscus* sp. 3. However, the Maximum Likelihood result is not very well supported (78.2/39).

*Spherillo* sp. was found to be most closely related with *Spherillo grossus* under both Maximum Likelihood, and Bayesian analysis. This analysis is well supported under Maximum Likelihood (87.8/92) but only someone supported under Bayesian analysis (0.595).

## *B.* ***Morphology***

### ***Material examined***

China: 1♂Holotype, Guangxi Province, Daxin County, 4.VII.2021.

### ***Description***

Fig. 7 *Spherillo sinoculus* n. sp. A, living specimen (dorsal view).

Dimensions: 6.62 x 3.36 mm. Pale white color and broad lack of pigment both alive and after preparation in ethanol, this is shown consistently in every part of the body with no variation in those prepared in ethanol. Live specimens show brown to light orange line running from the middle of the first pereonite to the first pleonite. Cuticles slightly translucent. Complete lack of eyes. Cephalon possesses frontal shield which does not clearly protrude over the vertex. In frontal view, the lateral sides of the frontal shield are triangular in shape, but slightly curved. Pereonite 1 with anterior corners slightly upturned. Posterior lateral corners rounded, while distal lateral margin curved. Pereonites 2 and 3 caudally bent and possess rounded triangular epimera. Pereonites 4-7 have quadrangular epimera which are slightly bent caudally. Telson about as long as it is wide and is square shaped, with distal margin rounded.

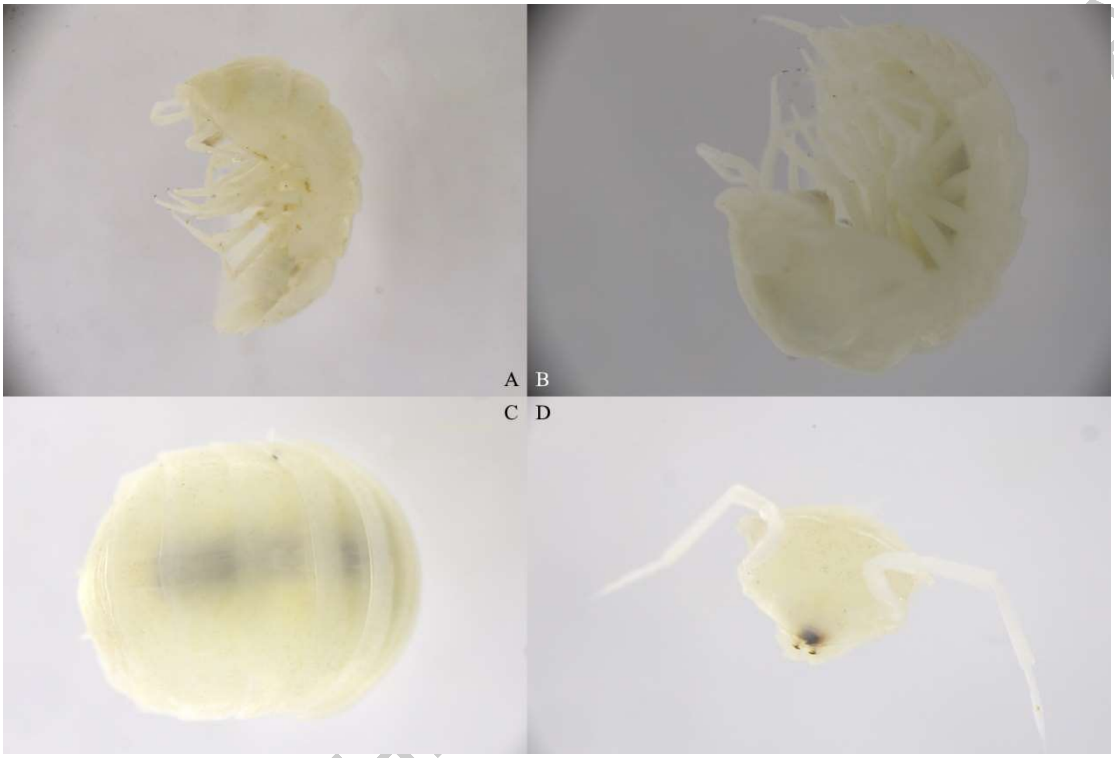

Fig. 8. *Spherillo sinoculus* n. sp. A and B, male side view; C, dorsal view; D, cephalon(frontal view).

Antennules possess three articles. The proximal article is slightly longer than the distal article, with the middle article being the shortest, about 1/4 the length of the proximal article. The middle article resembles a trapezium in shape, with the width being shorter where it joins the distal article. The distal article is slightly curved, and possesses a continuous line of short aesthetascs at its apex. Antennae reach the caudal margin of the first pereonite. Peduncle significantly longer than flagellum. First segment of flagellum is about 1/3 of the size of the second segment. The joint between the two segments are not obvious. The second segment of the flagellum tapers in diameter at the middle, and resembles a spindle. All pereopods short but slender, ungual setae extremely small and short, dactylar setae absent. All pereopods of roughly same length, though the carpus and propodus of pereopod 7 is about twice as long as that of the others. Long and flagelliform setae are concentrated on the side which is facing the body. The merus possesses 4, the carpus 4, and the propodus 8. Short setae line the sides of the carpus and propodus which faces away from the body, as well as the sides of the basis and ischium which face towards the body.

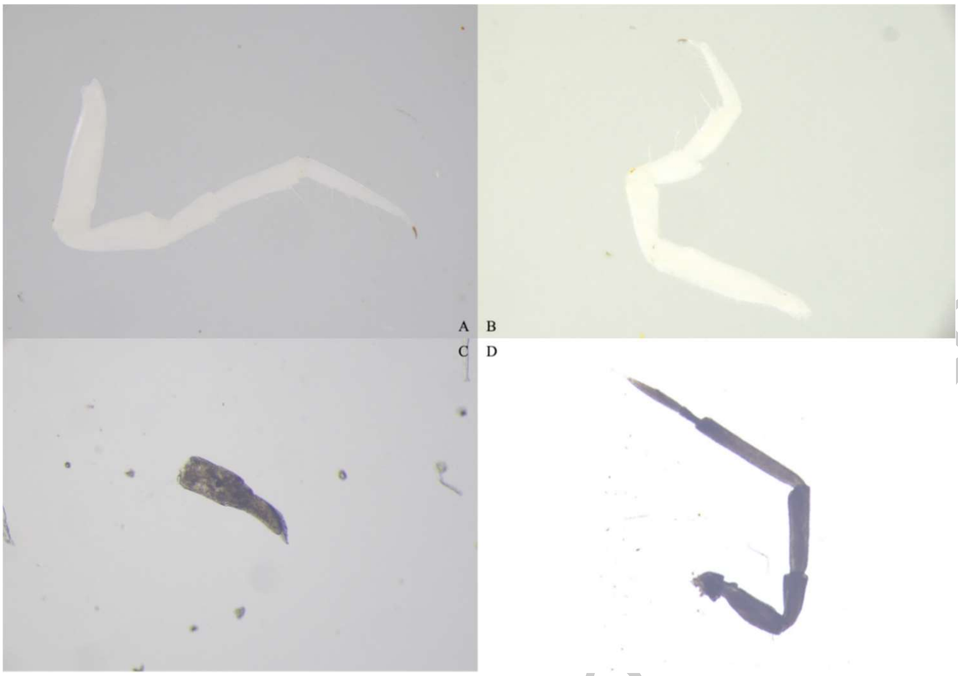

Fig. 9 *Spherillo sinoculus* n. sp. A, pereopod 1 (ventral view); B, pereopod 1 (dorsal view); C, antennule; D, antenna.

Mandibles are smooth and dark brown to black in color at the extremities and the teeth. The left mandible consists of four teeth, while the right mandible consists of three. Epipod of the maxilliped possess two short setae and one longer seta. Palp of the maxilliped possess two long setae at its base, and two short and narrow setae at its distal portion.

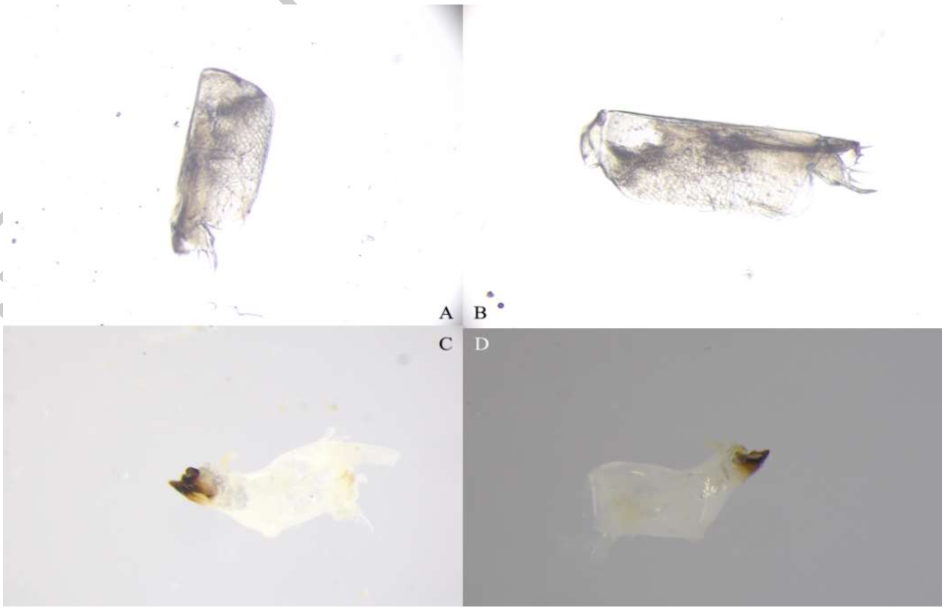

Fig. 10 *Spherillo sinoculus* n. sp. A, left maxilliped; B, right maxilliped; C, left mandible; D, right mandible.

Pleopod 1 endopod resembles the shape of a boomerang, with the distal portion narrower than the proximal portion. The distal portion is unrounded. Short and narrow setae line both lateral edges. Pleopod 1 exopod long and articulated, lacks setae.

Fig. 11. *Spherillo sinoculus* n. sp. A, endopod 1; B, exopod 2; C, exopod 1.

### *Distribution*

China (Longzhou, Chongzuo, Guangxi)

### *Diagnosis*

The most noticeable differences between *Spherillo sinoculus* and other species of its genus is its lack of pigmentation and eyes. See discussion below for more details.

### *Etymology*

This species is named after its lack of eyes by combining the two Latin roots *sine*- and -*oculus*, which respectively mean without, and eye. The particular way the roots are combined is also a play on words, as *sino* derives from the Latin *Sērēs*, which refers to China, the country in which this species was discovered.

# IV. DISCUSSION

## *A. The phylogenetic relationships between Guangxi Oniscidea species*

In this study, we analyzed the phylogentic relationships between different Guangxi Oniscidea along with related groups of non-Chinese Oniscidea. Our results show a multi-origin of cavernicolous Oniscidea, the need to revise genera within Oniscidea, and combined with morphology, a possible new species of cavernicolous Oniscidea.

The close genetic relationships between the rock face species *Burmoniscus* sp. 1 and the non-cavernicolous *Burmoniscus* sp. 2, as well as the relatively close genetic relationships between the troglobite *Spherillo sinoculus* and the non-cavernicolous *Spherillo* sp. suggests that cave dwelling behavior evolved independently across different lineages. This is supported by evidence for independent colonization of caves by Philosciidae oniscids in Hawaii (Rivera 2002). The specific niche partitioning and reason for cave colonization between different cavernicolous Oniscidea, however, is still left unresolved.

The monophyly of *Spherillo* being put into question is new, though not unexpected, as concerns have been raised regarding the monophyly of Oniscidea and genera inside of it as well (Lins 2017, Dimitriou 2018). A monophyletic *Spherillo* based on the Maximum Likelihood analysis would need to reclassify *Spherillo dorsalis*, and either revise *Merulana helsiana*, or remove *Spherillo obscurus* and *Spherillo sinoculus*. A monophyletic *Spherillo* based on Bayesian Analysis would either only include *Spherillo grossus* and *Spherillo* sp., or revise *Merulana helsiana* as well as *Cubaris murina*.

In addition to this, the monophyly of *Burmoniscus* is also put into question, with *Burmoniscus* sp. 1 and *Burmoniscus* sp. 2 forming a group, *Burmoniscus* sp. 3 forming a group, and *Burmoniscus meeusei* forming a group. These three groups are phylogenetically distinct and occupy very different parts of the Oniscidea phylogram. The first group is most closely related to Armadillo officinalis, the second group most closely related to *Trachelipus aegaeus*, and the third group most closely related with *Chaetophiloscia elongata*. All three of these species belong to different families, being Armadillidae, Trachelipodidae, and Philosciidae.

With the still field of oniscid phylogeny still burgeoning, the extreme polyphyly of these two genera might suggest the necessity of taxonomic reform, especially when it comes to troglobites, which are often described through morphology alone (Taiti and Gruber 2008, Taiti and Xue 2012).

### *B.* ***The comaprative characteristics of Spherillo sinoculus n. sp.***

Though the phylograms constructed using available data demonstrate *Spherillo sinoculus* to be most closely related to other species of the genus *Spherillo*, forming a sister taxon with them, it remains possible that *Spherillo sinoculus* could be more closely related to other species without genetic data. Considering this, this paper will also compare *Spherillo sinoculus* morphologically to other known true troglobites discovered in Guangxi (Beron 2015, Taiti and Gruber 2008, Taiti and Xue 2012)

#### ***Lucasioides cf. daliensis***

*Lucasoides cf. daliensis* possess eyes while *Spherillo sinoculus* possess no eyes. *Lucasoides cf. daliensis*' antennule setae are concentrated at the distal margin while *Spherillo sinoculus'* antennule setae line the lateral edge of its antennule as well.

#### ***Troglodillo latellai***

*Troglodillo latellai* possess eyes while *Spherillo sinoculus* possess no eyes. The first antennae of *Troglodillo latellai* are long, and reach the sixth pereonite, while the first antennae of *Spherillo sinoculus* are short and only reach the caudal margin of the first pereonite. The antennule setae of *Troglodillo latellai* are few and concentrated on the distal margin, while *Spherillo sinoculus*' antennule setae are many and line the lateral edge of its antennule as well.

#### ***Dryadillo guizhouensis***

*Dryadillo guizhouensis* possess eyes while *Spherillo sinoculus* possess no eyes. The antennule setae of *Dryadillo guizhouensis* are few and concentrated on the distal margin, while *Spherillo sinoculus'* antennule setae are many and line the lateral edge of its antennule as well.

### *Dryadillo parviocellatus*

*Dryadillo guizhouensis* possess eyes while *Spherillo sinoculus* possess no eyes. The carpus of *Dryadillo parviocellatus* possess many short along its ventral surface, while the carpus of *Spherillo sinoculus* do not.

### *Exalloniscus troglophilus*

*Exalloniscus troglophilus* is dorsoventrally flattened while *Spherillo sinoculus* is not. The uropods of *Exalloniscus troglophilus* are long, about the same length as its pleon, while the uropods of *Spherillo sinoculus* are short, and sit flush with the pleotelson. The first antennae of *Exalloniscus troglophilus* are long, and reach the caudal margin of the second pereonite. The first antennae of *Spherillo sinoculus* are short, and only reach the caudal margin of the first pereonite. *Exalloniscus troglophilus* is not able to conglobate, while *Spherillo sinoculus* is.

### *Trogloniscus hengliensis*

*Trogloniscus hengliensis* is dorsoventrally flattened while *Spherillo sinoculus* is not. The uropods of *Trogloniscus hengliensis* are long, about the 4/5 the length of its pleon, while the uropods of *Spherillo sinoculus* are short, and sit flush with the pleotelson. The first antennae of *Trogloniscus hengliensis* are long, and reach the caudal margin of the second pereonite. The first antennae of *Spherillo sinoculus* are short, and only reach the caudal margin of the first pereonite. *Trogloniscus hengliensis* is not able to conglobate, while *Spherillo sinoculus* is.

### *Trogloniscus deharvengi*

*Trogloniscus deharvengi* is dorsoventrally flattened while *Spherillo sinoculus* is not. The uropods of *Trogloniscus deharvengi* are long, about the 4/5 the length of its pleon, while the uropods of *Spherillo sinoculus* are short, and sit flush with the pleotelson. The first antennae of *Trogloniscus deharvengi* are long, and reach the caudal margin of the second pereonite. There is a width difference where the first pleonite joins the first pereonite in T *Trogloniscus deharvengi*, the width transition is smooth with *Spherillo sinoculus*. The first antennae of *Spherillo sinoculus* are short, and only reach the caudal margin of the first pereonite. *Trogloniscus deharvengi* is not able to conglobate, while *Spherillo sinoculus* is.

### *Trogloniscus clarkei*

*Trogloniscus clarkei* is dorsoventrally flattened while *Spherillo sinoculus* is not. The uropods of *Trogloniscus clarkei* are long, and protrude from the pleon, while the uropods of *Spherillo sinoculus* are short, and sit flush with the pleotelson. The first antennae of *Trogloniscus clarkei* are long, and reach the caudal margin of the second pereonite. There is a width difference where the first pleonite joins the first pereonite in Trogloniscus clarkei, the width transition is smooth with *Spherillo sinoculus*. The first antennae of *Spherillo sinoculus* are short, and only reach the caudal margin of the first pereonite. Trogloniscus clarkei is not able to conglobate, while *Spherillo sinoculus* is.

### *Trogloniscus trilobatus*

*Trogloniscus trilobatus* is dorsoventrally flattened while *Spherillo sinoculus* is not. The uropods of *Trogloniscus trilobatus* are long, and protrude from the pleon, while the uropods of *Spherillo sinoculus* are short, and sit flush with the pleotelson. *Trogloniscus trilobatus* possess three protrusions from its head, while *Spherillo sinoculus* doest not. *Trogloniscus trilobatus* is not able to conglobate, while *Spherillo sinoculus* is.

Considering this, the key characteristics of *Spherillo sinoculus* are its lack of eyes and pigmentation, compounded with its ability for conglobation and lack of dorsoventral flattening.

## V. CONCLUSION

This paper finds that cavernicolous behavior in Guangxi Oniscidea most likely arose multiple times independently. Additionally, the genera *Spherillo* and *Burmoniscus* are likely polyphyletic, and are in need of revision. *Spherillo* is likely split between four genera. *Spherillo dorsalis* allies with the genus *Cubaris*, *Spherillo grossus* and *Spherillo* sp. with the genus *Merulana*, and *Spherillo obscurus* and *Spherillo sinoculus* n. sp. each occupy relatively basal positions, but are more closely related to the *Merulana* group. *Burmoniscus* is likely split between three groups, all very distantly related. A new species, *Spherillo sinoculus*, was also found, with its key characteristics being its lack of eyes and pigmentation, compounded with an ability for conglobulation and absence of dorsoventral flattening.

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# ACKNOWLEDGMENTS

I am indebted to my three instructors, Mr. Edmund Donovan, Mr. Jiusi Yang, and Dr. Zhuqing He, who respectively gave valuable input for the paper, accompanied and helped me collect species in Guangxi, and taught me how to perform molecular and morphological phylogenetic analyses along with their theoretical components, while also extracting many genes for me to use in the paper. I would additionally like to thank my parents for their support, especially my father, who came with me to Guangxi.