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A New Species of Pit Mite (Trombidiformes: Harpirhynchidae) from the South American Rattlesnake (Viperidae): Morphological and Molecular Analysis

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Abstract

Background: Mites of the genus *Ophioptes*, parasitize a wide range of snakes' species worldwide. Pit mites develop in capsules inside the connective tissue or scales of their hosts and all stages have a genital-anal opening with no connection to the midgut. To this date, there are 15 known species, of which five occur in the Neotropical region. In South America four species have been described from Colubrid snakes.

Methods: Mites were collected from the chin shields and infralabial area of the head, and the anterior third portion of the snake. Comparisons of South American species of pit mites are provided for identification purposes. SEM imaging and illustration were made to provide morphological details of the new species. DNA extraction, sequencing, and phylogeny inference were performed of the new mite species and other species of Trombidiformes mites found on reptiles and amphibians.

Results: *Ophioptes ekans* n. sp. is described from the pits made by the mite on the scales and skin of a South American rattlesnake (*Crotalus durissus terrificus*) in Campo Limpo Paulista, São Paulo state, Brazil, captured on January 2014. The Genbank accession numbers of the new species are KU891263, KU891264 and KU891265. DNA sequences were used for molecular phylogenetic inference. Three nymhpal stages were observed for this species.

Conclusion: This is the first record of a viper snake from the sub-family Crotaline parasitized by *Ophioptes* mites. Molecular analyses showed that molecular systematic of Trombidiformes mites is still unclear and more sequences and other genes are needed do better elucidate the relationships within the group. These are the first DNA sequences (18rRNA V4 region) of mites from the Ophioptinae subfamily.

Keywords: *Ophioptes*; New species; Pit mite; Rattlesnake; Brazil

Introduction

Mites of the genus *Ophioptes*, are also called pit mites due to the pit-like lesions produced over the scales and connective tissue of their hosts (reptiles), which are solely snakes (Colubridae, Dipsadidae and Elapidae) [1-10]. These mites develop under the skin or scales of their hosts and adults emerge and reproduce over the body of the snake. Life cycle of these mites is divided in 4 phases egg, larva, nymph (both legless stages), and adults [7]. The development of immature stages occurs in the soft tissues and at the base of the scales. Larvae and nymphs are legless [1,7,11].

Ophioptes mites belong to the family Harpirhynchidae (permanent and highly specialized parasites of birds and snakes). The subfamily Ophioptinae parasitizes snakes of the families: Colubridae, Dipsadidae, Elapidae, and Lamprophiidae (Ophidia: Colubroidea). All development of these mites proceeds within the scales. The ancestor of Ohioptinae probably migrated from passerine birds onto the colubroid snakes [12].

To this date, the genus *Ophioptes* includes 15 species [7-10]. Of these, five species occur in the New World, four of them described parasitizing South American snakes (*O. parkeri* Sambon, 1928 in Bolivia, Brazil, and Argentine; *O. tropicalis* Ewing, 1933 in Guiana; *O. longipilis* and *O. brevipilis* Lizaso, 1981 in Brazil). The species *O. dromicus Allerd*, 1958 was described from Cuba.

In the present study, we described a new species of *Ophioptes* parasitizing the South American rattlesnake *Crotalus durissus terrificus* Laurenti, 1768 (Crotalinae: Viperidae), from the State of São Paulo, Brazil. Molecular data was produced and those DNA sequences from GenBank of some species of Trombidiformes mites found on reptiles and amphibians were used to infer phylogenetic relationships of these mite groups and for barcoding purposes.

Materials and Methods

Mite collection and preparation

During triage procedure at the herpetology section of the Laboratório Especial de Coleções Zoológicas, Instituto Butantan, a female adult South American rattlesnake was found infested with mites that were either moving slowly over the animal or embedded in the skin or scales of the anterior portion (Figure 1A). Embedded mites were collected via delicate scarification technique [10]. Mites were preserved in 100% ethanol before mounting in Hoyer's medium over slides. The specimens were clarified using hydroxyl potassium 5%, acetic acid 10% and lacto-phenol, to eliminate the guanine mass (common in this genus) that clouds the posterior portion (idiosoma) of the mites in all the stages of development [7]. Another group of mites was prepared and used for scanning electron microscopy (SEM) under QUANTA 250 SEM microscopes in the Laboratório de Biologia Celular, Instituto Butantan. Drawings of the mites were made using a LEICA DM 4000 B microscope and measurement with a LEICA DM 2500 microscope and Leica Application Suite Version 3.3.0 software. The leg and idiosomal chaetotaxy of the species description follows Grandjean [13,14] adapted by Kethley [15]. Palpal setation terminology follows Fain [16], and Grandjean [17] with adaptations proposed by Bochkov [18]. All measurements are given in micrometres (μm) and were taken according to the standard method [19,20].

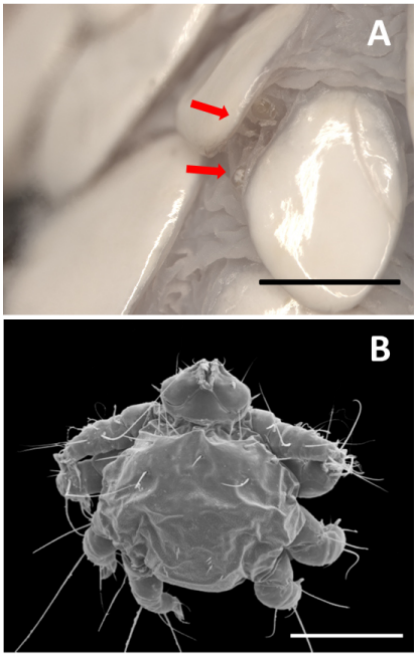

Figure 1: *Ophioptes* sp. n. A. Image in stereomicroscope of mites embedded beneath the scales of the infralabial area of the head of a female *Crotalus durissus terrificus*. B. SEM of female *Ophioptes ekans* n. sp., dorsal view. Scale bars: A 1800 μm, B 100 μm.

DNA extraction, PCR reactions and sequencing

We tested different protocols of DNA extraction (2 Quiagen based protocols, boiling protocol and guanidine isothiocyanate lysis) to assess what was the most suitable protocol for Trombidiformes mites. The DNA samples were subjected to polymerase chain reaction (PCR) amplification of mite 18S region V4 rRNA gene (forward primer-ATATTGGAGGGCAAGTC TGG; reverse primer-TGGCATCGTTTATGGTTAG) [21]. PCR products of the desired size (~480 bp) were purified and sequenced in an automated sequencer (ABI Prism 310). The nucleotide sequences were deposited in the GenBank database, and the accession numbers of the new species are KU891263, KU891264 and KU891265 (Table 1).

Phylogenetic inference (Barcoding)

The newly generated sequences were aligned with sequences previously deposited of other Trombidiformes mites available in GenBank (Table 1) using ClustalW [22] and were adjusted manually with GeneDoc program [23]. The alignment was used to construct phylogenetic trees using maximum parsimony and maximum likelihood with MEGA 6.06 [24] applying 500 bootstrap replicates, and Bayesian analysis was performed with MrBayes v.3.1.2 [25] with 2,000,000 generations, using a HKY+R model. The first 25% of the trees represented 'burn-in', and the remaining trees were used to calculate Bayesian posterior probabilities.

Ethical approval

The animals were caught and manipulated accordingly to the recommendations of the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA), and it was approved by the Ethics Committee on Animal Experimentation (CEUA) of the Universidade de São Paulo (FMVZ-USP) - no 3069/2013.

Results

Family HARPIRHYNCHIDAE Dubinin, 1957

Subfamily Ophioptinae Southcott, 1956

Genus *Ophioptes* Sambon, 1928

Ophioptes ekans n. sp. Mendoza-Roldan & Barros-Battesti

Description

Female: (Holotype Figure 1B) Body, including gnathosoma, 370 long (range 360-380 in 2 paratypes) and 439 wide (435-480). Gnathosoma 123 long (100-125), about 100 wide. Palps 55 long (50-60) and 122 wide (110-130). Palpal setae dF, dG (Puffed setae), and l"G pectinate, subequal in length, 17-22 long; IT setae 16-17 long (Figures 2A-D, and 3B). Subcapitulum ventrally with setae n 39-40 long, setae m bulked and 13-14 long. Idiosoma saccate, and smooth, 355 long (320-340) (Figures 3A, 4 and 5A-B). Genital-anal opening situated ventrally near the apex of the idiosoma, with three pairs anal-genital setae (spicules), g1-g3, and four pairs of genital setae,h1,h2,f1,f2 (22-23 long) around the genital-anal opening (Figures 2E, 3A and 4). Nautalae (1a,3a setae) 16-17 long; 1c,3c and 4c setae 17-19 long. Dorsal idiosoma setal lengths: Scx setae 11-12, seven pairs of dorsal-anterior setae (vi,ve,si,se,c3,c1,c2), subequal in length 46 (43-50) long, four pairs of dorsal-posterior setae (d1,d2,e1,e2) 12 (11-13) long. Setation of legs I-IV (respectively, including solenidia): Coxa 1(1c)-1(3c)-1(4c)-0; trochanter 1 (v)-1(v)-2 (l,v)-2 (l,v);); femur 2 (d,v)-1 (V)-1 (v)-0; genu 3 (l',d',v')-3 (l',d',v')-0-0; tibia 3 (d,l',v')-2 (l',d')-2 (l',d')-2 (l',d')-2 (l',d'); tarsus 10 (tc',tc",p'p",a',a",u',u",vs,ω)-7

(tc',p',a',u',u",vs,ω)-5 (tc',p',a',u',vs)-5 (tc',p',a',u',vs). Solenidia ω I and II bulked and short, about 15 long (Figures 2D and 3C).

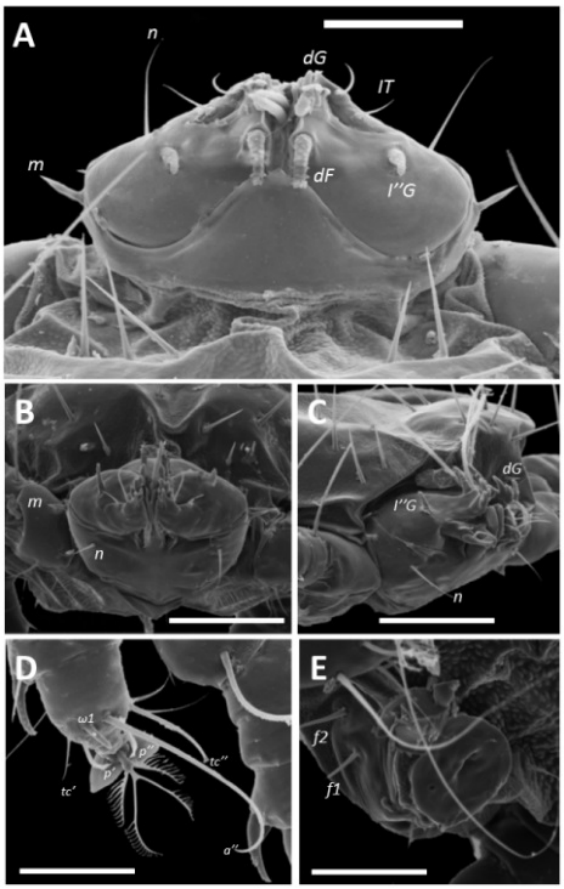

Figure 2: Different views of a female of *Ophioptes ekans* n. sp. under SEM. A. setae of the gnathosoma, dorsal view: B. gnathosoma, frontal view; C. gnathosoma, lateral view; D. Setae of the tarsus of leg I; E. genital plate. Abreviations: dF: tibial ventral setae; dG: foliate setae; m: latero-basal setae; n: ventro-basal setae; IT: tarsal anterior setae, I"G lateral palpal setae; (a", p', p", tc', tc", ω1): tarsal setae of leg I; f1, f2: posterior genital setae. Scale Bar: A, 50 µm; B, 50 µm; C, 40 µm; D, 40 µm.

Male: (2 paratypes, Figures 3B, C and 5A)-Body, including gnathosoma, 357-108 long, 324-339 wide (Figure 5A). Gnathosoma 81-90 long, 108-110 wide. Palpal setae dF, dG (puffed setae), and l^2G pectinate, subequal in length, 18-22 long (Figure 3B). Subcapitulum ventrally with setae n 24-27 long, and setae m 13-14 long. Idiosoma saccate, dorsally smooth, 228-230 long. Ventral surface of idiosoma with few transverse striations, without scales Genital-anal opening situated dorsally, posterior to the gnathosoma, with four pairs of genital setae, h1, h2, f1, f2 (12-13 long) around the genital-anal opening (Figure 5A). Ventrally Nautalae (1a, 3a setae) 13-14 long; 1c, 3c and 4c setae 24-25 long. Dorsal idiosoma setal lengths: Scx setae 12-13, seven pairs of dorsal-anterior setae (vi, ve, si, se, c3, c1, c2), subequal in length 24-30 long, two pairs of dorsal-posterior setae (d1, d2) 10-11 long. Setation of legs I-IV (respectively, including solenidia): Coxa 1(1c)-1(3c)-1(4c)-0; trochanter 1 (v)-1(v)-2 (l,v)-2 (l,v);); femur 2 (d, v)-1 (V)-1 (v)-0; genu 3 (l', d, v') - 3 (l', d', v')-0-0; tibia 2 (l', v')-2 (l', d')-2 (l', d')-2 (l', d'); tarsus 7 (tc', p', a', u', u", vs, ω)-7 (tc', p', a', u', u", vs, ω)-5 (tc', p', a', u', vs)-5 (tc', p', a', u', vs). Solenidia ω I and II bulked and short, about 13 long (Figure 3C).

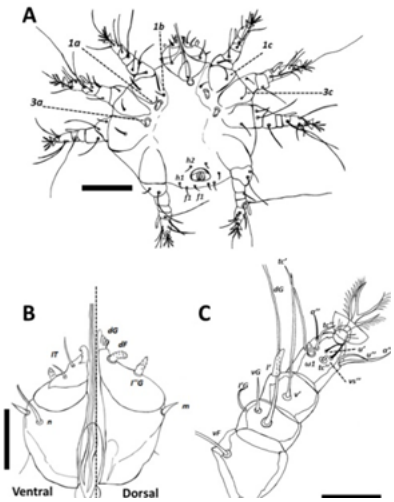

Figure 3: Illustrations of *Ophioptes ekans* n. sp. A. female idiosoma ventral view; B. gnathosoma, ventral and dorsal view of male and female; C. leg I of male and female. Abreviations: dG: apical foliate seta; 1b: anterior setae; 1a, 3a: nautalae; h1, h2, f1, f2: genital setae; m: latero-basal setae; IT: tarsal anterior setae; I"G: tibial dorsal setae; dF: ventral setae; n: ventro-basal setae. Scale bar: A, 100 µm; B, 50 µm; C, 50 µm.

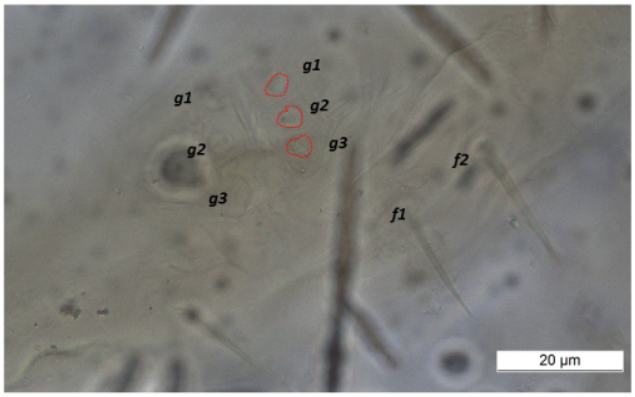

Figure 4: Ventral view of female *Ophioptes ekans* n. sp. showing genital-anal opening with three pairs of genital-anal setae Abreviations: g1-g3: genital-anal setae; f1-f2: genital posterior setar. Scale bar: 20 µm.

Nymphs: (2 paratypes Figure 4) Tritonymph-(Figure 6A)-Inside a shedding membrane surrounded by the capsule, with vestigial legs. Genital-anal opening absent. Body, including gnathosoma, 456 long, 426 wide. Gnathosoma 41 long. Measurements of the puparium or nymphal ecdysis-475 long and 486 wide. Deutonymph-(Figure 6B). The nymphal ecdise surrounding the deutonymph has complete gnathosoma and rounded idiosoma, with developed legs. Genital-anal opening present. Body, including gnathosoma, 394 long, 411 wide.

Gnathosoma n setae 27 long. Measurements of the puparium or nymphal ecdysis-443 long and 443 wide. Deutonymph next stage is possibly a male.

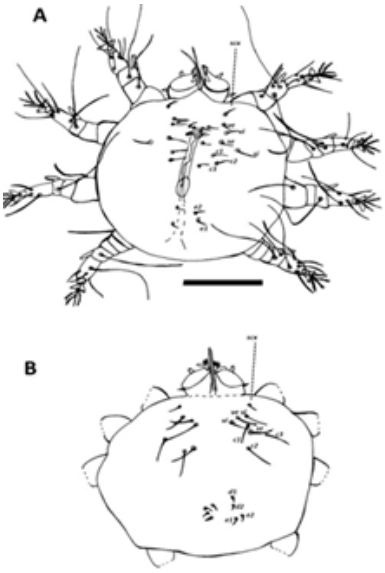

Figure 5: Dorsal illustrations of male and female *Ophioptes ekans* n. sp. A. idiosoma dorsal setae of male; B. idiosome setae of female. Abreviations: ve, vi, se, si, c1-c3: dorsal anterior setae; e1-e2: dorsal posterior setae; scx: scapular setae; Scale bar: A, B, 100 µm.

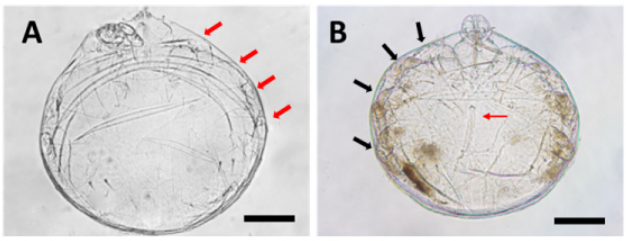

Figure 6: Tritonymph and deutonymph of *Ophioptes ekans* sp. n. A. Tritonymph in ventral view inside the capsule or puparium, red arrow are showing the vestigial legs in formation. B. Deutonymph in dorsal view inside the capsule or puparium, with developed legs (black arrows), and penis (red arrow) scale bar: A, B 100 µm.

Abnormalities: In some individuals, setae f2 unpaired. Males have absent dT setae.

Type material: Female holotype, (IBSP 12078) 2 female, 2 male and 2 nymphs paratypes (IBSP 12079), from a single female specimen of Crotalus durissus terrificus (Linnaeus, 1758) (Crotalinae: Viperidae, (IBSP 85008) Brazil: Campo Limpo Paulista, State of São Paulo, County, FL, 23°12′ 29.854 ″ S, 46°47′ 21.728 ″ W, 6 January 2014, coll. J. Mendoza-Roldan. The entire type series is deposited in the Acarological collection of the Laboratório Especial de Coleções Zoológicas of the Insituto Butantan, São Paulo, State of São Paulo, Brazil. The Type host is deposited in the Herpetological collection of the same laboratory of the Instituto Butantan.

Etymology: The species epithet is derived from the fictional character named "Ekans" a purple, serpentine Pokémon from the Pokémon Universe that resembles the type host the mites were infesting, and is a noun in apposition.

Differential diagnosis: The new species belongs to the "*parkeri*" group, which gathers all the neotropical species (species of this group have vF III present). The new species differs from the other five species known in the "*parkeri*" group, *O. brevipilis*, *O. dromicus*, *O. longipilis*, *O. parkeri*, and *O. tropicalis* by the presence in all stages of long n setae (2 to 3 times longer than in other species), and by 3 pair if genital-anal setae in females (Figure 4). The new species is closest to *O. parkeri* from Brazil, Bolivia and Argentine due to their similar size and leg chetotaxy. *Ophioptes ekans* n. sp. differs from *O. parkeri* species due to the body lengths, including gnathosoma of the male and female, are 357-559 and 360-380 and, respectively (*vs.* 330-350 and 380-390 long in *O. parkeri*). Leg Chetotaxy from *Ophioptes ekans* n. sp. is tarsus (10-7-5-5) in female and (7-7-5-5) in male; tibia (3-2-2-2) in female and (2-2-2-2) in male; genu (3-3-0-0); femur (2-1-1-0); trochanter (1-1-2-2); coxa (1-1-1-0) [*vs.* leg chetotaxy of *O. parkeri*: tarsus (10-10-8-8); tibia (3-3-2-2); genu (3-3-0-0); femur (2-1-1-0); trochanter (1-1-2-0)].

Remarks: Bochkov et al. proposed two different groups of *Ophioptes* species: "*parkeri*" group and "*schoutedeni*" group. Species of "*parkeri*" group are distributed in the neotropical region and *O. ekans* n. sp. belongs to this group, that is characterized by having a ventral branched seta on femur of leg III (Vf III). The "*schoutedeni*" group occurs on other continents (Africa, Eurasia) [12]. Morphologically they are having similar characteristics, and *O. parkeri* is the type species of the genus and of the sub family Ophioptinae. The new species differs from those species by females having three pairs of genital-anal setae in the "nidification organ" or genital-anal opening, different from other species that have four pairs (Figures 3E and 4). Furthermore, it is the only species of the South American species with three pairs of genital-anal setae on the "nidification" organ. Southcott and Fain described the genus as having 4 pairs of genital-anal setae, nevertheless they mentioned that there are exceptions like *O. najae* Fain, 1962 has 3 pairs of thorns, *O. lycodontis* Fain, 1964 that has no genital-anal setar, and *O. longipilis* can have unpaired of genital-anal setae [4,7].

Comparative material: *Ophioptes* brevipilis HOLOTYPE-Female (IBSP 6327) from Goiânia, state of Goiás, Brazil, 30.III.1979, Chironuis flavolineatus Jan, 1863. Paratypes-1 female and 4 males (IBSP 6202) from Colatina, state of Espírito Santo, Brazil, 17.II.1978, *Liophis poecilogyrus* Wied-Neuwied 1825; 9 females and 4 males (IBSP 6299), from Tupã, state of São Paulo, Brazil, 1.XII.1978, *Mastigodryas bifossatus* (Raddi, 1820); 1 male (IBSP 6351) from Uraí, state of Paraná, Brazil, 11.IX.1979, *Philodryas olfresii* (Lichtenstein, 1823).

Ophioptes longipilis HOLOTYPE Female (IBSP 6070) from Itú, state of São Paulo, Brazil, 07.II.1978, *Oxyrophus trigeminus* Duméril, Bibron & Duméril 1854 Jan, 1863. Paratypes-1 female and 4 males (IBSP 6202) in Colatina, state of Espírito Santo, Brazil, 17.II.1978, *Liophis poecilogyrus* Wied-Neuwied 1825.

Ophioptes parkeri 2 Males (IBSP 6205) from Araçoiaba da Serra, state of São Paulo, Brazil, 27.II.1978, *Chironius foveatus* Bailey 1955-18 Females and 9 Males (IBSP 6204) from Birita-Mirim, state of São Paulo, Brazil, 20.ll.1978, *Erythrolamprus aesculapii* Linnaeus 1766-7 Females and 9 Males (IBSP 6266) from Inuíba Paulista, state of São Paulo, Brazil, 22.IX.1978, *Erythrolamprus aesculapii* Linnaeus 1766-5

Females and 2 Males (IBSP 5981) from Presidente Wenceslau, state of São Paulo, Brazil, 14.IV.1976, *Erythrolamprus poecilogyrus* (listed as *Leimadophis poecilogyrus*) (Cope 1862).

Ophioptes tropicalis LECTOTYPE-1 Female (U.S.N.M. No. 1081.) from British Guiana (collected at Washington D.C.), 1931, *Chironius carinatus Linnaeus* 1758 (listed as *Erpetodryas carinatus*).

Molecular phylogeny and barcoding

For the phylogenetic analyses, the following sequences obtained from this study (in bold) and those withdrawn from the GenBank database were used (Table 1). Phylogenetic trees inferred in this study were compared to those of previous studies that also used the Chelicetara 18S V4 gene [21]. Furthermore, morphological phylogenies of the Trombidiformes order were used to validate and confirm the relationships between the families and superfamilies of the mites [26,27]. The produced trees were obtained using Maximum Likehood (ML), Maximum Parsimony (MP) and Bayesian methods. In all the methods, the scorpion Androctonus australis was used as outgroup. Bayesian analyses showed the most congruent results (Figure 7). Mites sequenced in the present study, showed strong relationships and were grouped by the three analyses in the "supercohort" Eleutherengonides [with 2 representative superfamilies: Cheyletidea (*Ophioptes* and *Demodex*) and Raphignathoidea (*Geckobia*)], and in the Cohort Parasitengona (Leeuwenhoekiidae: *Hannemania*, Trombiculidae: *Fonsecia*, Trombidiidae and Erythraeidae: *Erythrites* and *Erythroides*). Within the order Trombidiformes, as in previous studies using the V4 region of the 18S ribosomal gene, the relationships between the groups were not well supported and therefore, did not corroborate the morphological systematic division. Nevertheless, Trombidiformes was well separated from Mesostigmata (Figure 7) [28-30].

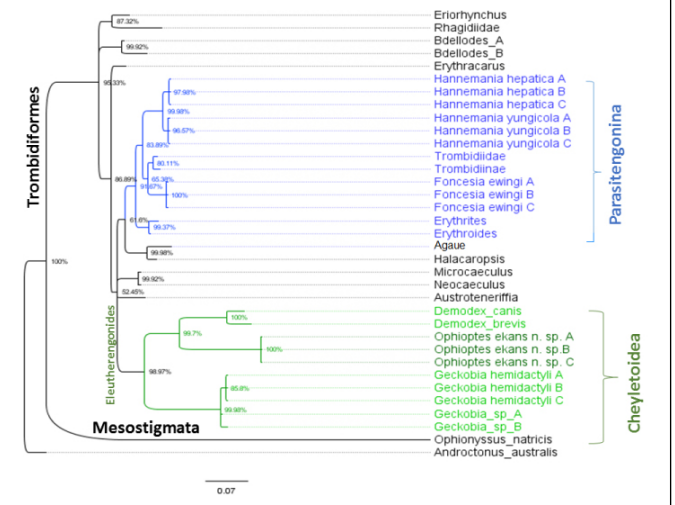

Figure 7: Bayesian Phylogenetic tree of the Trombidiformes species of mites based on the parcial sequences of the 18S V4 rRNA gene. A total of 35 mite sequences and A. australis sequence as outgroup. Number of nodes correspond to the posterior probability value of 2.000.000 generations tree.

Discussion

Sambon and Fain [1,7] succinctly presented the life cycle of pit mites. It is, until this date, known that larva and nymphs are legless stages. In the present study two other nymphal stages were described (tritonymph and deutonymph) and this stages had legs or early formation of legs.

The different nymphal instars of *O. ekans* n. sp. suggest that in fact, there is a protonymph (legless stage) reported by Sambon and Fain [1,7], a tritonymph (instar with vestigial leg formation, without sexual differentiation), and a deutonymph (instar with leg development and sexual dimorphism, inside the cocoon or puparium). It is presumed that this genus develops entirely inside the capsule or puparium and only emerges when it is sexually active. These species of pit mite are unable to excrete; therefore, they gather the detritus as guanine masses inside the idiosoma. This probably results in a short adult lifespan. This kind of life cycle is also seen in other Cheyletoidea mites, such as *Demodex* species, which lifespan after the adult emerges from the capsule is very short, around 120 hours [31-34].

Although *Ophioptes* species live inside the skin, there are no studies or descriptions of the impact that the lesions caused by these mites may have on the overall health status of their hosts. Nevertheless, cavities (pits) were observed on the skin of the examined snakes during the present study. Presumably, pits can act as an entrance door for bacterial infections and other opportunistic pathogens, by loss of connective tissue integrity that can lead to dysecdysis (improper shedding of the skin). Hence, retained skin can cause secondary infections that if not treated in time, can cause mortality [35-37].

Regarding parasite specificity, *Ophioptes* species were recorded parasitizing colubrid and elapid ophidians worldwide [1-10]. Furthermore, Lizaso described two species of *Ophioptes* (*O. brevipilis* and *O. longipilis*) parasitizing colubrids snakes in Brazil [10]. However, the author only worked with non-venomous snake fauna. *Ophioptes ekans* n. sp. is the first mite of this genus ever described in viper snakes. The new species shows that the Ophioptinae subfamily has an ancestral origin in the Colubriodea superfamily. Last cladistic studies suggested that pit mites might have originated when their ancestors passed from birds to snakes that predated on them. In some cases, such as elapid snakes, pit mites ancestors passed from colubrid snakes to elapid snakes by their ophidophagal behaviour [38-40]. Crotalus snakes and the vast majority of vipers do not have birds as part of their diet, nonetheless, as being part of the Colubroidea super family, explains pit mite parasitism on them [41-43].

Phylogenetic analyses with the 18S V4 rRNA gene, showed low node and branch supports within the order Trombidiformes. The majority of the relationships between cohorts and families did not corroborate the last cladistic morphological analyses [26,27]. Previous studies of Otto and Wilson and Pepato et al. showed similar results regarding phylogenetical relationships within groups of this order. Nonetheless, the three analyses performed in the present study showed congruent grouping of the Parasitengona cohort (Trombiculidae, Leewenhoekiidae, Trombidiidae and Erythraeidae) [21,44]. However, relationships between those groups remain vague and confusing. Trombiculidae mites appeared to be more related to Trombidiidae mites, than with Leeuwenhoekiidae, that was previously thought to be a subfamily of Trombiculidae [36,45,46].

Chelicerata					
Acari	**Suborder**	**Family**	**species**	**Genbank Code**	**IBSP ID**
Trombidiformes	Prostigmata	Anystidae	Erythrocarus	AF142109	-
		Bdellidae	Bdellodes A	AF142118	-
		Bdellidae	Bdellodes B	AF142119	-
		Caeculidae	Microcaeculus	AF142110	-
		Caeculidae	Neocaeculus	AF142111	-
		Demodicidae	Demodex brevis	HQ727999	-
		Demodicidae	Demodex canis	HQ727998	-
		Eriorhynchidae	Eriorhynchus	AF142116	-
		Erythraeidae	Eryhrites	AF142105	-
		Erythraeidae	Erytrhoides	AF142106	-
		Halacaridae	Aguae	AF142107	-
		Halacaridae	Halacaropsis	AF142108	-
		Harpirhynchidae	O. ekans n. sp. A	KU891263	12079
		Harpirhynchidae	O. ekans n. sp. B	KU891264	12079
		Harpirhynchidae	O. ekans n. sp. C	KU891265	12079
		Leeuwenhoekiidae	H. hepatica A	KU891269	12050
		Leeuwenhoekiidae	H. hepatica B	KU891270	12015
		Leeuwenhoekiidae	H. hepatica C	KU891271	12058
		Leeuwenhoekiidae	H. yungicola A	KU891272	12049
		Leeuwenhoekiidae	H. yungicola B	KU891273	12049
		Leeuwenhoekiidae	H. yungicola C	KU891274	12048
		Pterygosomatidae	Geckobia A	AF142113	-
		Pterygosomatidae	Geckobia B	AF142114	-
		Pterygosomatidae	G. hemidactyli A	KU891266	12084
		Pterygosomatidae	G. hemidactyli B	KU891267	12086
		Pterygosomatidae	G. hemidactyli C	KU891268	12087
		Rhagidiidae	Undetermined	AF142117	-
		Teneriffidae	Austoteneriffia	AF142115	-
		Trombidiidae	Undetermined	AF142123	-
		Trombidiidae	Undetermined	GQ864280	-
		Trombiculidae	F. ewingi A	KU891275	12071
		Trombiculidae	F. ewingi B	KU891276	12071
		Trombiculidae	F. ewingi C	KU891277	12071
Mesostigmata	-	Macronyssidae	Ophionyssus natricis	FJ911853	-
Arachnida	-	-	-	-	-
Scorpiones	-	Buthidae	A. australis	X74761	-

Table 1: Species of Chelicerata, whose sequences were used for the phylogenetic analyses of the 18S rRNA V4 region gene.

On the other hand, the *Ophioptes* species of the family Harpirhynchidae and the *Geckobia* species of the family Pterygosomatidae, grouped within the Eleutherengonides supercohort, and *Ophioptes* and *Demodex* showed high supports and grouped in the Cheyletoidea superfamily [47]. The monophyly of the Trombidiformes was not corroborated, due to the low number of sequences used that cannot elucidate if this group in fact is monophyletic. Dabert et al. and Pepato et al. showed that Trombidiformes was paraphyletic [30,44]. The region of the ribosomal gene used in the present study showed to be highly conserved, at a level that most of the fragments of the sequences are identical in the mites and in the scorpion outgroup species. Otto and Wilson reported this homology as well [21]. Therefore, its important in future studies to analyze other regions of the 18S rRNA gene to determine which would be the most informative region of this gene. Nonetheless, this study contributed 15 more sequences of Trombidiformes mites that allowed including other families in the phylogenetic analysis and barcoding for future studies. In the molecular systematic, the assessment of homologies between characters are made through sequence alignment. This alignment however, is more a computational matter than a biological one, thus efforts are focused on formulating algorithms that can by means of similarity criteria, evaluate nucleotide homologies. Nonetheless, it would be advisable to create better and more efficient methods that can align nucleotides according to their evolutionary ancestry [48,49].

Conclusions

Ophioptes ekans sp. n. Is the first species of this genus of pit mites that parasitizes vipers (Crotalus durissis terrificus), and to date, South America has six known species of *Ophioptes* mites (all of the "*parkeri*" group). The life cycle of the new species has three nymphal instars, and at least two of them have leg development. Origins of the parasitism of this genus are clearly from ancestors of the Colubroidea super family. Molecular sequences of this new species of *Ophioptes* are the first sequences for this genus as well as other 13 sequences of reptile Trombidiformes mite sequences. Molecular systematic of Trombidiformes mites is still very unclear and more sequences and other genes are needed do better elucidate the relationships within the group.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

DMBB conceived the study, described the new species, and revised it critically for important scientific and intellectual content. RB-S and FCJ helped to prepare the specimens for morphological and molecular studies. AM and FAN-B collaborated in the sequence alignment and phylogenetic analyses. FLF identified the host and helped with the mite collection; and all the authors read, reviewed and approved the submitted version.

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