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# A new species of the genus *Eutrombicula* Ewing, 1938 (Trombidiformes: Trombiculidae) and new records for the species *Eutrombicula batatas* (Linnaeus, 1758) in Brazil

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## ABSTRACT

The genus *Eutrombicula* consists of more than 80 known species worldwide. Species of this genus parasitize amphibians, reptiles, birds and mammals. In the present study, we describe *Eutrombicula daemonei* n. sp. collected on the social flycatcher, *Myiozetetes similis* (Passeriformes) from southeast region of Brazil and partial sequence of the 18S gene was obtained for this mite species. In addition, we provide new locality records and hosts for *Eutrombicula batatas* (L.) in Brazil.

**Keywords** new records; chigger mites; bird; host; *Myiozetetes similis*; parasite; Brazil

**Zoobank** <http://zoobank.org/DC1A4B29-31D8-498C-8F9F-63A12E4E7C13>

## Introduction

Currently, the genus *Eutrombicula* Ewing, 1938, is represented by more than 80 species worldwide (Stekolnikov and González-Acuña 2010). Species of this genus have been recorded as parasites of amphibians, reptiles, birds and mammals (Hoffmann 1990). Thirty-seven species of the genus have been recorded from South America; of these, five species were listed from Brazil (Jacinavicius *et al.* 2018). Brazil is divided into five administrative regions: north, northeast, central-west, southeast and south (Contel 2014). *Eutrombicula bruyanti* (Oudemans, 1910) was recorded from the southeast region of the country, while *Eutrombicula goeldii* (Oudemans, 1910) and *Eutrombicula tinami* (Oudemans, 1910) were described from Brazil without specific locality information (Oudemans 1910). However, Jacinavicius *et al.* (2018) recorded *E. tinami* from the state of São Paulo, in the southeast region. The other two species found in Brazil are *Eutrombicula alfreddugesi* (Oudemans, 1910) and *Eutrombicula batatas* (Linnaeus, 1758). Specimens of *E. alfreddugesi* were recorded from the central-west region in

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the states of Goiás (Carvalho *et al.* 2006) and Mato Grosso (Fonseca 1932); from the northeast region in the states of Bahia (Menezes *et al.*, 2009; Delfino *et al.* 2011) and Maranhão (Faccini *et al.* 2017); and in the southeast region in the states of Minas Gerais (Carvalho *et al.* 2006) and São Paulo (Fonseca 1932). *Eutrombicula batatas* was recorded from the north region, in the states of Amazonas (Ewing 1925) and Pará (Ewing 1925; Bequaert 1926); from the northeast region in the state of Maranhão (Faccini *et al.* 2017); from the central-west region in the state of Mato Grosso (Confalonieri and Benez 1976); and from the southeast region in the state of Rio de Janeiro (Confalonieri and Carvalho 1973).

A new species of *Eutrombicula* is described based on a larva found on a social flycatcher, *Myiozetetes similis* (Aves: Passeriformes: Tyrannidae) collected in Fazenda Volta Grande, Santa Bárbara do Monte Verde Municipality, Minas Gerais state located in the southeast region of Brazil and a partial sequence of the 18S gene was obtained for this species. In addition, new records of localities and hosts for *E. batatas* in Brazil are provided.

## Materials and methods

### Collection of mites and morphological study

Eight specimens (holotype and paratypes) of a chigger (Acari: Trombidiformes: Trombiculidae) were found on one specimen of a bird species, *Myiozetetes similis* (Passeriformes: Tyrannidae), collected with a mist net in Fazenda Volta Grande, Santa Bárbara do Monte Verde (21°58'S, 43°41'W, elevation 736 m), Minas Gerais State, Brazil, in September of 2014. The bird was identified using Ridgely and Tudor (2009). Of the material collected and stored in ethanol 100%, two specimens were mounted on slides in Hoyer's medium according to Walter and Krantz (2009) for identification and were deposited in the Acari Collection of the Instituto Butantan (IBSP), four specimens were prepared according to Walter and Krantz (2009) for scanning electron microscopy (SEM) which were coated with gold. After imaging, the specimens were removed from the stubs with acetone and stored in alcohol and deposited in the IBSP collection. The SEM micrographs were obtained with a Digital Scanning Microscope FEI, Quanta 250, located at the Laboratório de Biologia Celular, Instituto Butantan. In addition, two specimens were retained for molecular analysis.

Drawings and measurements of the new species were made with a Leica DFC 500 digital camera. Extended focal range images were composed with Leica Application Suite version 2.5.0. All the measurements are in micrometers ( $\mu\text{m}$ ), followed by the mean and standard deviation. The holotype measurements are highlighted. The images were prepared with Adobe Photoshop v. 13.0, and Inkscape V.2.

Other species from the genus *Eutrombicula* deposited at the IBSP collection were examined for comparison with the new species. The specimens were identified using Brennan and Goff's (1977) key to the genera and Brennan and Reed's (1974) key to species of Venezuela, as well as original descriptions of the species of the genus *Eutrombicula*. For species with incomplete descriptions, such as *Eutrombicula bruyanti* (Oudemans, 1910), *Eutrombicula batatas* (Linnaeus, 1790), *Eutrombicula goeldii* (Oudemans, 1910) and *Eutrombicula tinami* (Oudemans, 1910), we used redescriptions by Oudemans (1912) and Jenkins (1949) and illustrations by Brennan and Reed (1974).

Terminology generally follows Goff *et al.* (1982) with additions and modifications by Stekolnikov (2008), Stekolnikov and Daniel (2012). The nomenclature for specialized and opisthosomal setae follows Kethley (1990), Wohltmann *et al.* (2006, 2007) and Bassini-Silva *et al.* (2017).

### Molecular analysis

In order to enrich genetic banks with chiggers sequencing and to provide future works, two fragment of DNA were tested. The DNA of two specimens of *Eutrombicula daemioni*

n. sp., was extracted using the Guanidine Isothiocyanate lysis protocol (Chomczynski 1993). Each mite was placed in an Eppendorf microtube, and punctured in the idiosomal region with a sterile needle (1.20 \* 40 – 18G). After DNA extraction, the exuvia were recovered, mounted on slides, and kept as vouchers. A conventional PCR targeting a ≈500-pb fragment of the 18S ribosomal RNA gene was performed using primers Mite18S-1F (3'-ATATTGGAGGGCAAGTCTGG-5') and Mite18S-1R (3'-TGGCATCGTTTATGGTTAG-5'), as described by Otto and Wilson (2001). Positive samples were pooled and subsequently subjected to a second PCR analysis attempted to target ≈560-680-pb fragment of the cytochrome oxidase I gene (COI) using primers bcdF01 (CATTTTCHACTAAYCATAARGATATTGG) and bcdR04 (TATAAACYTCDGGATGNCCAAAAAAA) following the protocol of Dabert *et al.* (2010) and Dabert *et al.* (2008), respectively, with modifications of Moniuszko *et al.* (2015). For each reaction, negative (Milli-Q water free of DNA), and positive controls. All PCRs were performed on a Mastercycler Gradient (Eppendorf® California, USA). PCR products with concentrations higher than 20 ng/μl, were selected and purified with ExoSap-IT (GE Healthcare Pittsburgh, PA). Sanger sequencing of the samples were performed in the “Centro de Pesquisa sobre Genoma Humano e Células Tronco do Instituto de Biociências da USP”. Obtained sequences were assembled with Sequencing Analysis 5.3.1 software and submitted to BLAST analyses (Altschul *et al.* 1990) in order to infer similarities with that of other mites available in GenBank. Different haplotypes were visually discriminated after an alignment using CLUSTAL W algorithm (Thompson *et al.* 1994) implemented in Geneious R9 (Kearse *et al.* 2012).

## Ethical approval

The bird host of the new species was caught and manipulated in accordance with the recommendations of the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee on Animal Experimentation (CEEAA) of the Dean of the Universidade Federal de Juiz de Fora, MG (UFJF) – n°042/2012 (SISBio n° 29268-6).

## Results

### Morphological study

The description of *Eutrombicula daemonei* n. sp. is provided below and, in addition, the material previously deposited in the IBSP was identified as *Eutrombicula batatas*. These represent the first records of *E. batatas* parasitizing *Nyctibius griseus* (Nyctibiiformes: Nyctibiidae), *Didelphis aurita* (Didelphimorphia: Didelphidae) and *Cavia aperea* (Rodentia: Caviidae) from the following localities: São Paulo and Cotia municipality, São Paulo state; Wenceslau Braz municipality, Paraná state; Santa Cruz do Sul municipality, Rio Grande do Sul state and Tucuruí municipality, Pará state.

### *Eutrombicula* Ewing, 1938

*Eutrombicula* Ewing, 1938: 293; Loomis & Wrenn, 1984: 152; Domrow & Lester, 1985: 8; Hoffmann, 1990: 42; Stekolnikov & González-Acuña, 2015: 3.

Type species: *Microthrombidium alfreddugesi* Oudemans, 1910

### *Eutrombicula daemonei* n. sp. Bassini-Silva and Jacinavicius

Zoobank: [34647AB1-CA11-42AA-9F8A-1C527A9CC5B1](https://zoobank.org/34647AB1-CA11-42AA-9F8A-1C527A9CC5B1)

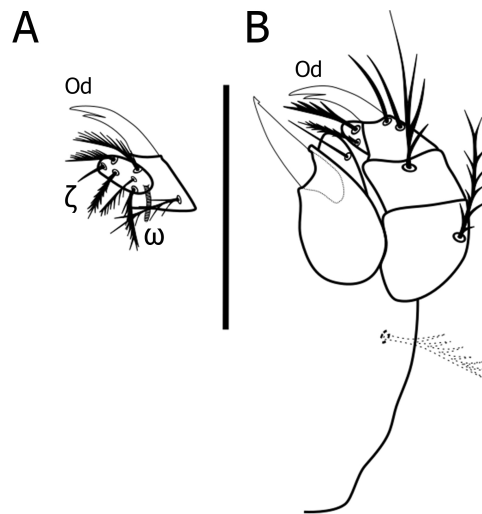
**Larva (Figures 1-5, Tables 1, 2 and 3)**

**Diagnosis**

Palptibia with the dorsal and lateral nude setae, and a ventral branched seta, palptarsus with seven branched setae (plus  $\omega$ ,  $\zeta$ ), galeal (adoral) setae nude, odontus bifurcated; the first row (C) of dorsal opisthosoma setae with 10 setae, the second and third rows (D and E) with 8 setae each; genu legs I each with three  $\sigma$ , tarsus of the legs I each with a  $\epsilon$  positioned distal to  $\omega$ , tibia of the legs III each with two mastisetae, tarsus of the legs III each with four mastisetae.

**Description**

**Gnathosoma** — palp setal formula B/B/NNB/7B $\zeta\omega$ ; odontus bifurcate; cheliceral blade with tricuspid cap; gnathobase punctate (Figs. 1A-B and 5D), galeal (adoral) setae nude (Fig. 1B).



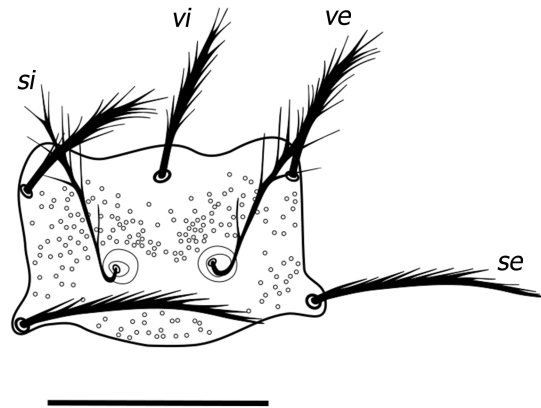
**Figure 1** Morphological details of *Eutrombicula daemonei* n. sp. A. ventral view of palp tarsus; B. dorsal view of gnathosoma. Symbols:  $\zeta$  = eupathidium of palp tarsus;  $\omega$  = solenidium of palp tarsus; Od = odontus. Scales: A and B 50  $\mu$ m.

**Idiosoma** — Eyes 2/2 set in an ocular plate, anterior eye larger; prodorsal sclerite (= *scutum*) with 1 pair of flagelliform trichobothria (*si*), 1 pair of *ve* (= AL), 1 pair of *se* (= PL) and a single *vi* (= AM) seta, *si* > *se* > *vi* > *ve*; all of the trichobothria with long and slender setules, but not so densely distributed; the anterior margin of the prodorsal sclerite undulating, and the lateral margins slightly concave posteriorly, posterior margin convex and the posterolateral corners are peninsulate each with a *se* seta (Figs. 2A and 5C). Sixty two to sixty six idiosomal setae, dorsal opisthosoma with 5 pairs of setae in the C row, with the *c5* pair of seta in an

**Table 1** Number of idiosomal setae of *Eutrombicula daemonei* n. sp. (Total = sum of all columns).

Identifications	C row	D row	E row	F & H rows	Sternals	Ventrals	Total
BSP 12374B (1) *	10	8	8	6	4	28	64
IBSP 12374B (3) *	10	8	8	6	4	30	66
IBSP 12374B (4) *	10	8	8	6	4	26	62

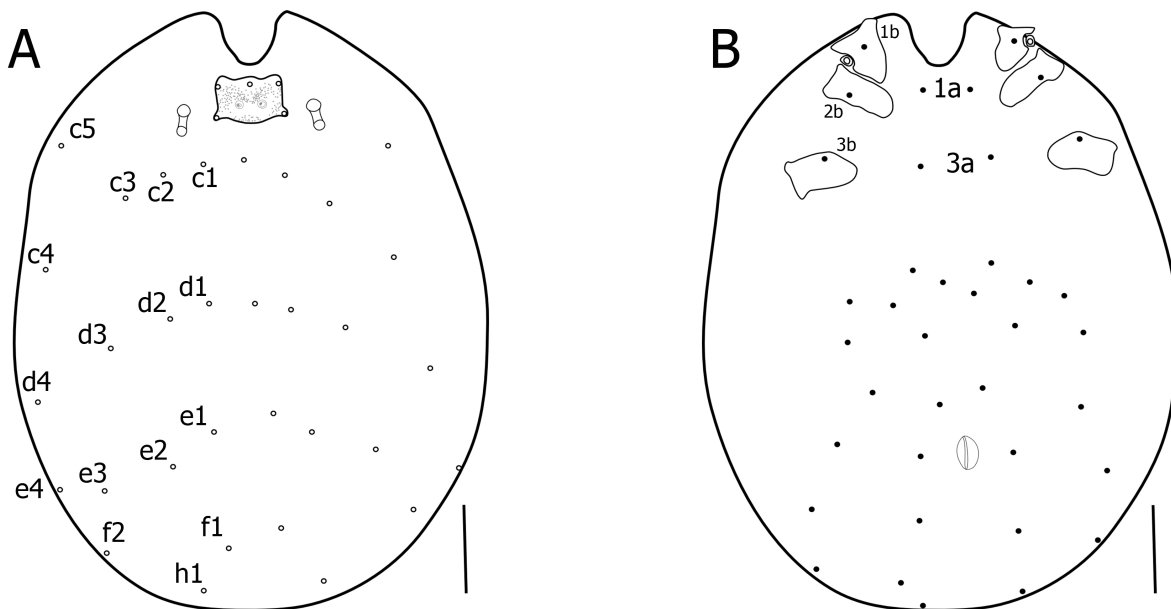
\*The number in parenthesis represents a collection code. The slide IBSP 12374B (1) is that of the holotype, IBSP 12374B (3) and (4) are paratypes. IBSP 12374B (2) is not represented in this table 1 because of a poor condition that do not allowed to get reliable setal measurements.



**Figure 2** Morphological details of *Eutrombicula daemonei* n. sp. prodorsal sclerite. Symbols: *ve* = anterolateral setae; *se* = posterolateral setae; *vi* = anteromedial seta; *si* = trichobothria. Scale: 50  $\mu\text{m}$ .

anterior position (= humeral setae), D row with 4 pairs of setae, E row with 4 pairs of setae, F row with 3 pairs of setae, the H row with a pair of *h1* setae, totaling 32 dorsal opisthosomal setae 2 pairs of sternal setae (1a, 3a), and 16 to 18 setae located anterior of the anus and 10 to 12 setae located posterior of the anus, totaling 26 to 30 ventral setae (Figs 3A-B, 5E and Table 1).

**Legs** — Femur legs I-III each divided into a basifemur and telofemur, each leg terminated



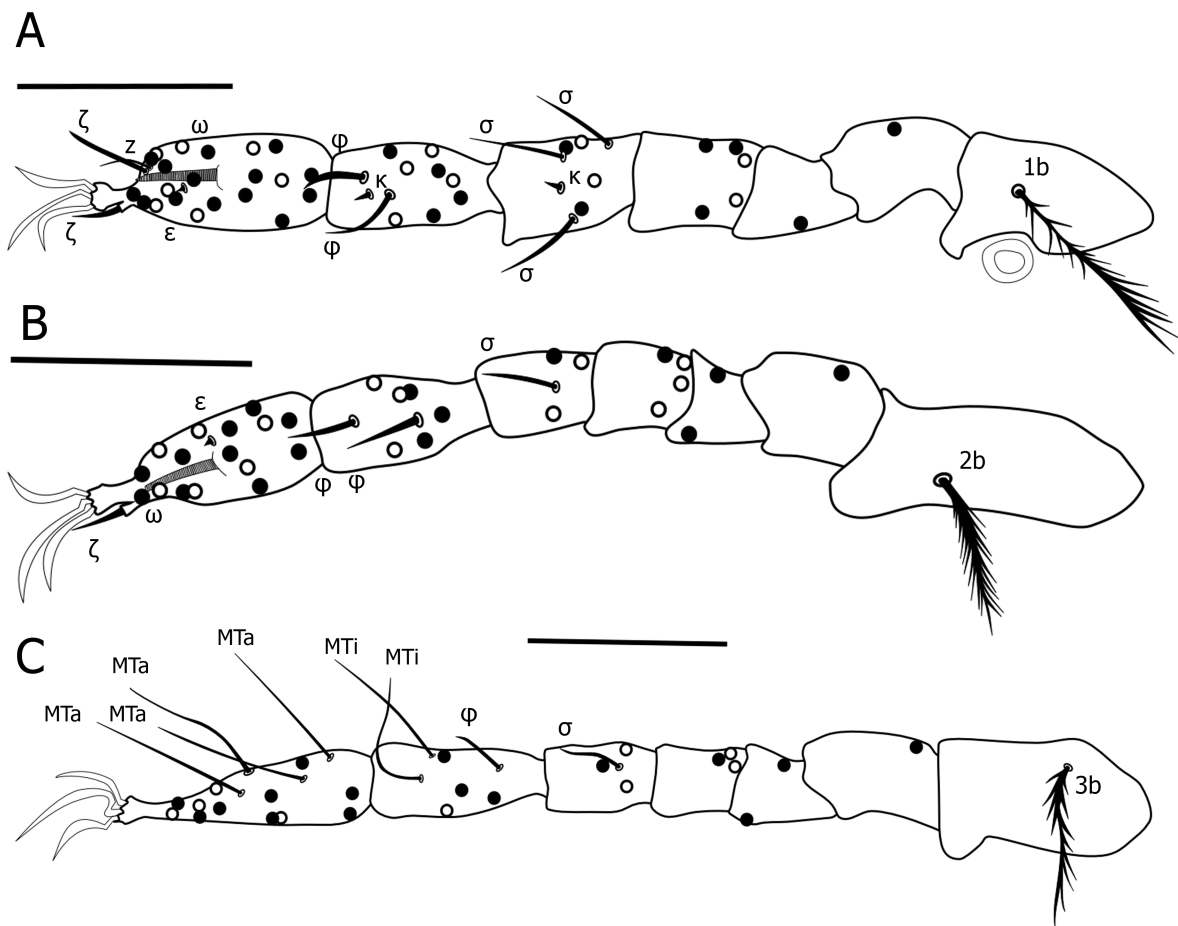
**Figure 3** Morphological details of *Eutrombicula daemonei* n. sp. A. dorsal view of idiosoma; B. ventral view of idiosoma. Black spots = ventral setae of the idiosoma; white spots = dorsal setae of the idiosoma. Symbols: *c1-c5* = setae of the C row; *d1-d4* = setae of the D row; *e1-e4* = setae of the E row; *f1-f2* = setae of the F row; *h1* = setae of the H row; 1a = anterior sternal setae; 3a = posterior sternal setae; 1b = seta of the coxal field of the leg I; 2b = seta of the coxal field of the leg II; 3b = seta of the coxal field of the leg III; Scales: A and B 100  $\mu\text{m}$ .

with a pair of claws and a claw-like empodium, without onychotriches, coxal fields not striate. **Leg I** — coxal field with 1 branched seta 1b (1B); trochanter 1B; basifemur 1B; telofemur 5B; genu 4B, 3  $\sigma$  with  $\kappa$ ; tibia 8B, 2  $\varphi$  with  $\kappa$ ; tarsus 21B, with  $\omega$ ,  $\epsilon$ , dorsal eupathidium ( $\zeta$ ) with a companion seta z and terminating with a subterminal eupathidium ( $\zeta$ ), famulus ( $\epsilon$ ) distal to  $\omega$  (Figs. 4A and 5A). **Leg II** — coxal field seta 2b (1B); trochanter 1B; basifemur 2B; telofemur 4B; genu 3B,  $\sigma$ ; tibia 6B, 2  $\varphi$ ; tarsus 15B, with  $\omega$ ,  $\epsilon$ , and a subterminal eupathidium ( $\zeta$ ), base of  $\epsilon$  proximal to  $\omega$  (Fig. 4B). **Leg III** — coxal field seta 3b (1B) on anterior margin, trochanter 1B; basifemur 2B; telofemur 3B; genu 3B,  $\sigma$ , tibia 4B, and 2  $\varphi$  mastisetae; tarsus 12B and 4 mastisetae (Figs. 4C and 5B).

**Type data** — (IBSP 12374B)

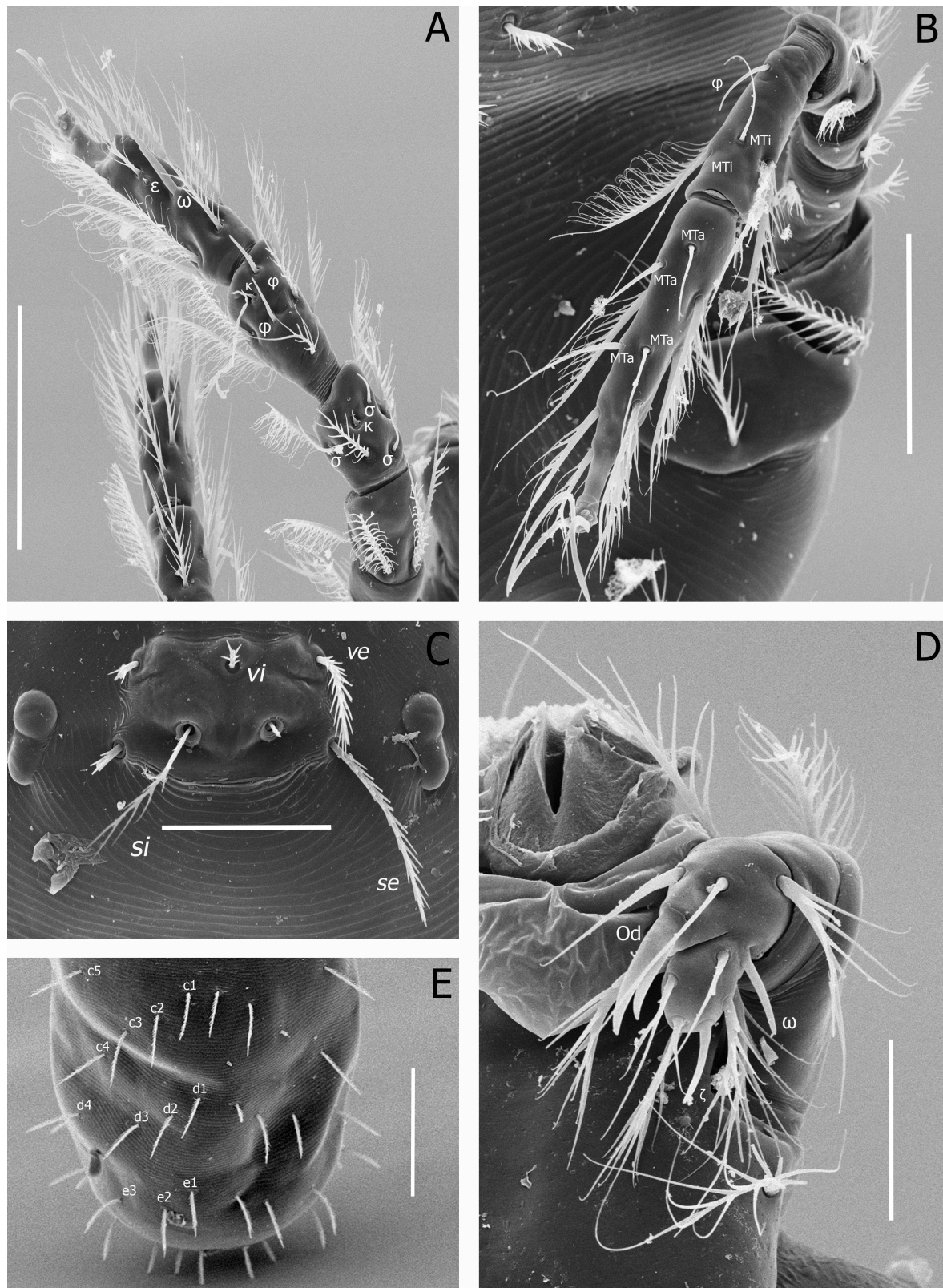
Holotype: larva, Fazenda Volta Grande, Santa Bárbara do Monte Verde municipality, Minas Gerais state (21° 58'S, 43° 41'W), 25-IX-2014, *Myiozetetes similis* (Passeriformes: Tyrannidae), Maturano, R. coll. Paratypes: seven larvae (IBSP 12374B), same data.

**Etymology** — The name is given in honor to Brazilian researcher Erik Daemon, in recognition of his work on ectoparasites.



**Figure 4** Morphological details of *Eutrombicula daemonei* n. sp. A. Leg I; B. Leg II; C. Leg III. Black spots = ventral setae of the idiosoma; white spots = dorsal setae of the idiosoma. Symbols:  $\sigma$  = solenidia on the genu of the legs I, II and III;  $\kappa$  = microsetae on genu and tibia of the leg I;  $\varphi$  = solenidia on the tibia of the legs I, II and III;  $\omega$  = solenidion on the tarsus of the legs I and II;  $\epsilon$  = famulus on the tarsus of the legs I and II;  $\zeta$  = dorsal eupathidium on the tarsus of the leg I, subterminal eupathidium on the tarsus of the legs I and II; z = companion seta on tarsus of the leg I; MTa = mastisetae on tarsus of the leg III; MTi = mastisetae on tibia of the leg III; 1b = seta of the coxal field of the leg I; 2b = seta of the coxal field of the leg II; 3b = seta of the coxal field of the leg III. Scales: A–C 50  $\mu$ m.





**Figure 5** Morphological details of *Eutrombicula daemoni* n. sp. A. leg I; B. leg III; C. prodorsal sclerite; D. palp tibia and tarsus; E. dorsal opisthosomal setae. Abbreviations and symbols: A –  $\sigma$  = solenidia on the genu of the leg I;  $\kappa$  = microsetae on genu and tibia of the leg I;  $\varphi$  = solenidia on the tibia of the leg I;  $\omega$  = solenidion on the tarsus of the leg I and  $\varepsilon$  = famulus on the tarsus of the leg I. B –  $\varphi$  = solenidion on the tibia of the leg III, MTi: masetisetae on tibia of the leg III and MTa: masetisetae on tarsus of the leg III. C – ve: anterolateral setae; vi: anteromedian seta; se: posterolateral setae and si: trichobothria. D – Od: odontus;  $\zeta$  = eupathidium of palp tarsus and  $\omega$  = solenidion of palp tarsus. E – c1-c5 = setae of the C row; d1-d4 = setae of the D row; e1-e3 = setae of the E row; scales: A 50 $\mu$ m, B-D 40 $\mu$ m, E 100 $\mu$ m.

**Table 2** Standard measurements of *Eutrombicula daemoni* n. sp. (n=4)

	AW	PW	SB	ASB	PSB	SD	P-PL	AP	vi	ve	se	si	cs	1a	3a	DMIN	DMAX	VMIN	VMAX	I	II	III	Ip	TaIII	TaW
Holotype	62	59	23	16	5	21	13	20	32	33	49	57	44	40	38	38	45	26	32	228	216	255	699	65	15
Minimum	59	56	22	14	5	21	10	20	32	30	44	54	43	40	38	36	40	25	30	198	180	222	642	59	13
Maximum	63	67	28	18	8	25	15	26	40	35	51	59	48	43	41	40	46	30	33	237	222	263	706	71	18
Mean	61	61	25	16	7	23	12	23	35	33	49	56	45	41	39	38	43	26	31	222	206	246	674	65	16
SD	1,4	3,8	2,1	1,1	1	1,7	1,5	2,4	2,3	2	2,1	1,7	1,8	1,1	1,2	1,2	2,3	1,9	0,8	12,8	13	13,4	24,7	3,2	1,3

Legend: AW = distance between the bases of the *ve* setae; PW = distance between the bases of the *se* setae; SB = distance between sensillary bases; ASB = distance from sensillary bases to extreme anterior margin of the prodorsal sclerite; PSB = distance from sensillary bases to extreme posterior margin of the prodorsal sclerite; SD = ASB + PSB; P-PL = distance from posterolateral to extreme posterior margin; AP = distance between the bases of *ve* and *se*; *vi* = anteromedial seta; *ve* = anterolateral setae; *se* = posterolateral setae; *si* = trichobothria; *cs* = humeral setae; 1a = anterior sternal setae; 3a = posterior sternal setae; DMIN = minimum length of dorsal opisthosomal setae; DMAX = maximum length of dorsal opisthosomal setae; VMIN = minimum length of ventral idiosomal setae; VMAX = maximum length of ventral idiosomal setae; I = length of leg I; II = length of leg II; III = length of leg III; Ip = sum of leg lengths (coxal field to tarsus); TaIII = length of tarsus of the leg III; TaW = width of tarsus of the leg III.

**Differential diagnosis**

The new species was compared with known Brazilian *Eutrombicula* species and with other South American species in the genus. *Eutrombicula daemoni* n. sp. can be separated from the other South American *Eutrombicula* species by the following combination of characters: six mastisetae on the leg III – two on the tibia and four on the tarsus (1F and 2B), and five pairs of dorsal opisthosomal C row setae. It is similar to *Eutrombicula batatas*, which has two mastisetae on the tibia of leg III and five pairs of setae (*c1-c5*) in C row on the dorsal opisthosoma, but differs in having four mastisetae on tarsus leg III compared to three in *E. batatas*. The new species, has a nude lateral palptibial seta, the inner prong smaller than the outer prong and four mastisetae on the tarsus of the leg III, whereas *Eutrombicula bruyanti* has a forked lateral palptibial seta the odontus with a long inner prong and a short outer prong arising in the middle part of shaft and bearing tarsus of the leg III with only one mastiseta. *Eutrombicula daemoni* n. sp. is similar to *Eutrombicula alfreddugesi* in having a branched ventral palptibial seta, but differs in having the inner prong of odontus arising in the middle part of shaft and four mastisetae on the tarsus of the leg III, whereas *E. alfreddugesi* has the bifurcation of outer prong of the odontus arises subapically to the inner prong, and only a single mastiseta on tarsus of leg III. The new species is also similar to *Eutrombicula tinami* in having branched ventral setae on the palptibia and the shape of the prongs of the odontus, but differs

**Table 3** Standard measurements of the specialized setae of *Eutrombicula daemoni* n. sp. (n=4)

	σ I	κ I	φ I	κ I	ω I	ε I	ζ I	σ II	φ II	ω II	ε II	ζ II	σ III	φ III	MTa	MTi
Holotype	15	3	13	3	17	3	13	16	12	11	3	15	20	21	43	48
Minimum	15	3	13	3	15	3	13	15	12	11	3	15	19	20	43	48
Maximum	16	3	14	3	19	4	16	17	13	12	4	17	20	21	46	49
Mean	16	3	14	3	17	3	14	16	12	11	3	16	20	21	44	49
SD	0,3	0,2	0,5	0,1	1,1	0,2	1	0,9	0,3	0,4	0,6	0,6	0,4	0,6	1,2	0,4

Legend: σ I = length of solenidia on genu of the leg I; κ I = length of microseta on genu of the leg I; φ I = length of solenidia on tibia of the leg I; κ I = length of microseta on tibia of the leg I; ω I = length of solenidion on tarsus of the leg I; ε I = length of famulus on tarsus of the leg I; ζ I = length of subterminal eupathidium on tarsus of the leg I; σ II = length of solenidion on genu of the leg II; φ II = length of solenidia on tibia of the leg II; ω II = length of solenidion on tarsus of the leg II; ε II = length of famulus on tarsus of the leg II; ζ II = length of subterminal eupathidium on tarsus of the leg II; σ III = length of solenidion on genu of the leg III; φ III = length of solenidion on tarsus of the leg III; MTa = length of mastisetae on tarsus of the leg III; MTi = length of mastisetae on tibia of the leg III.

in having two mastisetæ on the tibia of leg III (no mastisetæ on tibia leg III in *E. tinami*) and four mastisetæ on the tarsus of leg III (one mastisetæ in *E. tinami*). The shape of the odontus in *Eutrombicula goeldii* is similar to that of the new species, but *E. goeldii* has nude ventral setæ on the palptibia whereas in the new species this seta is branched. In addition, *E. bruyanti*, *E. alfreduggesi*, *E. tinami* and *E. goeldii* have 4 pairs (*c1-c4*) of setæ in the C row, differing from *Eutrombicula daemonei* n. sp. which has 5 pairs (*c1-c5*) of setæ in the C row.

We compared the new species to other South American *Eutrombicula* using Brennan and Reed's (1974) key to Venezuelan *Eutrombicula*. The three species most similar to *Eutrombicula daemonei* n. sp. were *Eutrombicula spipi* Brennan and Reed, 1974, *Eutrombicula vacillata* Brennan and Reed, 1974 and *Eutrombicula wolfenbarger* Brennan and Reed, 1974. These three species and the new species have mastisetæ in the tibia of leg III, but only the new species and *E. spipi* have 5 pairs (*c1-c5*) of setæ in the dorsal opisthosomal C row and three *sigma* on leg I [(*E. vacillata* has 6 pairs (*c1-c6*) of setæ and two *sigma* and *E. wolfenbarger* has 7 pairs (*c1-c7*) of setæ and two *sigma*]; however, *E. spipi* differs from the new species in having only two mastisetæ on the tarsus of the leg III.

Recently, Stekolnikov & González-Acuña (2015) described three new *Eutrombicula* species from Chile, *Eutrombicula nerudai* Stekolnikov & González-Acuña, 2015, *Eutrombicula mistrali* Stekolnikov & González-Acuña, 2015 and *Eutrombicula picunche* Stekolnikov & González-Acuña, 2015. We compared these with the new species. *Eutrombicula nerudai* and *E. mistrali* have one mastisetæ on the tarsus of the leg III, a nude seta on the palpgenu, and the outer prong of odontus arising subapically from inner prong, while *E. daemonei* n. sp. has four mastisetæ on the tarsus of the leg III, a branched seta on the palpgenu and the odontus arising in the middle part of shaft. *Eutrombicula picunche* and *E. alfreduggesi* differ from the new species by the same characters.

### ***Eutrombicula batatas* (Linnaeus, 1758)**

**New records** — 1 larva (IBSP 349D), Butantan, São Paulo municipality, São Paulo state, 09-VI-1935, “rodent” unidentified (Rodentia: Cricetidae); 1 larva (IBSP 350A), same locality, 06-VI-1935, “rodent” unidentified (Rodentia: Cricetidae); 2 larvae (IBSP 383), same locality, 09-X-1933, *Rattus norvegicus* (Rodentia: Muridae); 5 larvae (IBSP 484C), same locality, 29-X-1933, *Didelphis aurita* (Didelphimorphia: Didelphidae); 1 larva (IBSP 1690), same locality, *Crotophaga ani* (Cuculiformes: Cuculidae); 1 larva (IBSP 1647), Morro Grande, Cotia municipality, São Paulo state, 20-II-1939, “owl” unidentified (Strigiformes: Strigidae); 2 larvae (IBSP 1638), Wenceslau Braz municipality, Parana state, 03-III-1939, *Cavia aperea* (Rodentia: Caviidae); 2 larvae (IBSP 2128), Santa Cruz do Sul municipality, Rio Grande do Sul state, 04-XII-1951, *Hydrochoerus hydrochoeris* (Rodentia: Caviidae); 10 larvae (IBSP 13285), Tucuruí municipality, Pará state, no date, *Nyctibius griseus* (Nyctibiiformes: Nyctibiidae).

### **Molecular analysis**

Two samples (paratypes) of the new species were submitted to PCR and were positive to the 18S gene PCR and amplified two identical fragments of 409-bp (GenBank accession numbers: MG707783 and MG707784). BLAST comparisons of these sequences showed 99.7% (408/409-bp) of identity with homologous sequences from *Eutrombicula splendens* (KP325057) and 99.5% (407/409-bp) from *Quadrasetta brasiliensis* (MF113413; MF113412; KY934464). None of the samples amplified for the COI gene.

In addition, we obtained partial sequences for the 18S gene from the new species. These sequences are the first molecular identification of this genus for Brazil. At this time, it is still not possible to infer molecular differences between species that have a high similarity to *Eutrombicula daemonei* n. sp. based on an analysis of their 18S sequences, due to the slow rate of evolution of this gene, so different genera when compared have high similarity, which reflects that these species belong to a same family of mites.

Unfortunately, there are only these two species of *Eutrombicula* with 18S sequences deposited in Genbank. It is necessary to enrich the gene bank for species of this genus for future comparisons. Until now, we do not have recent material of *E. batatas* for molecular analysis and didn't possible comparisons with the new species.

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