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Complementary description of *Neoseiulus tunus* (De Leon) (Acari: Mesostigmata: Phytoseiidae) and observation on its reproductive strategy

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similarity between *N. tunus* and *N. neotunus*, the possible synonymy between them was mentioned in the literature (Moraes and Mesa, 1988; Lofego, 1998).

In laboratory colonies of populations identified as *N. tunus*, initiated with specimens collected from Campinas and Icém, São Paulo state, males were never observed (our unpublished observations). However, males have been found in field samples (wild population) as reported by Furtado *et al.* (2006) and in this publication. These findings suggest that *N. tunus* may undergo non-obligatory thelytokous parthenogenesis (Suomalainen, 1950; Meêus *et al.*, 2007).

Although pseudo-arrhenotoky (Schulten, 1985) is the most common parthenogenetic reproductive mechanism in phytoseiids, some species reproduce by thelytoky. Wysoki and Bolland (1983) confirmed thelytokous parthenogenesis in *Typhlodromus (Anthoseius) transvaalensis* (Nesbitt) and *Neoseiulus salish* (Chant & Hansell). Biological observations under laboratory conditions suggested the occurrence of thelytokous parthenogenesis also in *Amblyseius herbicolus* (Chant) (Van der Merwe, 1968) (reported as *A. largoensis* – see Muma & Denmark, 1970; Blommers, 1976) and *Phytoseius intermedius* Evans & Macfarlane (Azevedo *et al.*, 2016).

The objective of this study is to present morphological information about various Brazilian populations of *N. tunus*, with illustrations of the female and the male, as well as an evaluation of reproductive strategy of two populations of *N. tunus*. Another aim of this work was to evaluate the possible synonymy between *N. tunus* and *N. neotunus*.

MATERIALS AND METHODS

Taxonomy

The holotype females of *N. tunus* and *N. neotunus* were examined under phase contrast microscopy at FSCA, Gainesville, Florida, USA, where they are deposited. Additionally, specimens deposited in the mite collections of Departamento de Zoologia e Botânica, UNESP (Universidade Estadual Paulista), São José do Rio Preto and of Departamento de Entomologia e Acarologia, ESALQ-USP (Escola Superior

de Agricultura “Luiz de Queiroz”, Universidade de São Paulo), Piracicaba, both in São Paulo state, Brazil, were also examined under phase contrast microscopy. All specimens examined were from samples collected in field.

The classification system used was that of Chant and McMurtry (2007). Dorsal and ventral setal nomenclature were that of Rowell *et al.* (1978) and Chant and Yoshida-Shaul (1991), respectively. The notation for gland pores (solenostomes) or lyrifissures (poroids) are based on Athias-Henriot (1975). Measurements were done with the use of a graduate eyepiece. All measurements are given in micrometers. Illustrations were made using a drawing tube attached to the microscope.

Parthenogenesis

Tests were performed to verify the reproductive strategy of two populations of *N. tunus* under controlled conditions. The experiments were conducted at 28±1 °C, 80±10% relative humidity and 12 h of daily photophase, in a growth chamber.

Establishment of colonies

Colonies were established from mites collected from two sources:

– Campinas (CAMP): from bell pepper, *Capsicum annuum* L. (Solanaceae), placed in an urban forest fragment in Campinas, São Paulo state (22°52′04″S; 47°04′41″W).

– Icém (ICEM): from *Trichilia casareti* D. DC. (Meliaceae), growing naturally in a forest fragment at Icém, São Paulo state (20°20′48″S; 49°15′49″W).

About 300 predators were collected and used to establish the colony for each population. Colonies were maintained in rearing units similar to those described by McMurtry and Scriven (1965), consisting of a tray containing a foam mat overlaid by a plastic plate onto which the predators were placed. The foam mat was maintained permanently wet with distilled water, to prevent predators from escaping. Cattail pollen (*Typha domingensis* Persoon) and *Aleuroglyphus ovatus* (Troupeau) (Acari: Astigmatina: Acaridae) were used as food sources in both stock colonies, maintained in a room where environmental conditions were not controlled (24 – 27

°C, 60–80% relative humidity and about 12 h photophase).

Experimental procedure

Thirty newly hatched larvae of each *N. tunus* population were randomly taken from the respective stock colony and each was placed in an experimental unit. Each experimental unit consisted of a plastic container (2.6 cm diameter × 1.5 cm in height) whose base was covered with a layer of solidified paste of a mixture of gypsum and activated charcoal (9v: 1v) (Abbatiello, 1965). The edge of each unit was coated with insect glue to prevent the mites from escaping and water was added daily to the unit to maintain humidity high. Each predator was fed with an excess of larvae or protonymphs of *Aleuroglyphus ovatus* (Troupeau), added daily to each unit.

Units were evaluated every 24 hours for 12 consecutive days to determine adult emergence, pre-oviposition and oviposition. At each day, eggs obtained by the females of each population were put together into a new unit, feeding them as mentioned for the previous generation. They were maintained until reaching the adult stage to be sexed. Mite handling and observations were done under a stereomicroscope (magnification 30x).

RESULTS AND DISCUSSION

Taxonomy

Neoseiulus tunus (De Leon)

(Figure 1)

Typhlodromips tunus De Leon, 1967: 29; Moraes *et al.*, 1986: 151.

Amblyseius tunus McMurtry and Moraes, 1989: 181.

Neoseiulus tunus Lofego, 1998: 45; Chant and McMurtry, 2003: 21, 2007: 31; Moraes *et al.*, 2004: 148; Demite *et al.*, 2016.

Typhlodromips neotunus Denmark & Muma, 1973: 255 (new synonym).

Amblyseius neotunus Moraes and Mesa, 1988: 155.

Neoseiulus neotunus Chant and McMurtry, 2003: 21; 2007: 29; Moraes *et al.*, 2004: 135; Demite *et al.*, 2016.

Female.

Measurements given in Table 1.

Dorsum — Idiosomal setal pattern (Chant and Yoshida-Shaul, 1991): 10A:9B/JV-3:ZV. Dorsal shield reticulate, except for a smooth transverse band at level of Z4; with 15 pairs of lyrifissures (id1, idx, id2, id4, id6, is1, idm1, idm2, idm3, idx', idm4, idm6, idl1, idl3 and idl4) and seven pairs of pores (gd1, gd2, gd4, gd5, gd6, gd8 and gd9). Is not easy to see all pores and lyrifissures in an unique specimen. Setae *j1*, *j3*, *z2*, *z4*, *Z1*, *Z4*, *Z5*, *s4*, *S2* and *S4* serrate; *j4*, *j5*, *j6*, *z5*, *S5*, *r3* and *R1* lightly serrate and *J5* smooth.

Venter — Sternal shield with three pairs of setae and two pairs of lyrifissures; *st4* on metasternal plate. Genital shield smooth. With two pairs of metapodal plates. Ventrianal shield constricted between JV2 and anus levels, smooth; with three pairs of pre-anal setae (*JV1*, *JV2* and *ZV2*) and a pair of pores mesad of *JV2*. Four pairs of opisthogastric setae on unsclerotized cuticle (*JV4*, *JV5*, *ZV1* and *ZV3*). All ventral setae smooth, except *JV5* serrate.

Peritreme — Extending anteriorly to level of *j1*.

Spermatheca — Calyx cup-shaped; atrium inconspicuous.

Chelicera — Movable digit with three teeth in addition to apical tooth; fixed digit with five–seven teeth in addition to apical tooth and *pilus dentilis*.

Legs — Macrosetae smooth and with tiny distal knobs. Chaetotatic formula of genu II: 2-2/0-2/0-1 and of genu III: 1-2/1-2/0-1.

Male

(n= 10, all collected in this study).

Dorsum — Dorsal shield ornamentation as in female, 241 (230–255) long, 159 (150–171) wide at level of *s4*; setae: *j1* 19 (15–20), *j3* 22 (20–25), *j4* 14 (11–16),

TABLE 1: Measures of holotypes of *Neoseiulus tunus* (De Leon) and *N. neotunus* (Denmark & Muma) and measures (mean and range) of *N. tunus* females from several regions from Brazil (this work) and reported in other localities (previous works).

Character	This work			Previous works		
	<i>Neoseiulus tunus</i> * (Trinidad)	<i>Neoseiulus neotunus</i> * (Brazil)	Other Brazilian specimens (n= 40)	Moraes et al. (2000) (French Antilles)	Guanilo et al. (2008a) (Argentina)	Guanilo et al. (2008b) (Peru)
DSL	288	293	288 (270–315)	289 (277–297)	290 (275–300)	283 (280–285)
DSW	166	170	177 (168–192)	154 (150–156)	181 (175–185)	174 (170–178)
<i>j1</i>	23	24	24 (20–27)	23 (19–26)	23 (21–25)	22 (21–23)
<i>j3</i>	28	30	28 (25–31)	24 (19–27)	29 (28–30)	27 (25–29)
<i>j4</i>	13	12	14 (11–17)	14 (13–16)	14 (12–16)	14 (13–15)
<i>j5</i>	16	13	14 (11–16)	16 (14–18)	14 (14–15)	16 (15–18)
<i>j6</i>	20	19	19 (14–24)	19 (18–21)	20 (19–22)	20 (20–21)
<i>J2</i>	20	21	21 (16–27)	21 (18–24)	26 (22–28)	22
<i>J5</i>	7	7	8 (7–9)	9 (8–10)	8 (7–10)	7
<i>z2</i>	20	24	23 (19–27)	23 (21–24)	25 (24–27)	20 (20–21)
<i>z4</i>	30	38	31 (27–37)	27 (23–31)	35	27 (25–30)
<i>z5</i>	16	18	15 (11–18)	18 (16–19)	16 (15–16)	15 (15–16)
<i>Z1</i>	24	27	25 (21–31)	25 (24–26)	29 (26–32)	26 (25–27)
<i>Z4</i>	43	49	45 (41–54)	40 (39–40)	47	41 (40–41)
<i>Z5</i>	64	69	70 (63–80)	66 (64–68)	70 (67–72)	67 (65–68)
<i>s4</i>	36	38	38 (33–44)	35 (34–37)	42 (42–43)	37 (36–37)
<i>S2</i>	31	36	34 (29–40)	33 (32–34)	41 (38–43)	32 (31–33)
<i>S4</i>	19	22	20 (17–25)	20 (19–21)	25 (23–30)	20 (18–21)
<i>S5</i>	12	14	14 (10–20)	13 (11–16)	16 (17–20)	13
<i>r3</i>	24	22	25 (22–30)	21 (21–22)	26 (23–30)	23 (20–25)
<i>R1</i>	14	19	17 (14–20)	17 (16–18)	18 (17–20)	16 (15–16)
<i>st1-st3</i>	54	59	58 (55–62)	56 (55–56)	54 (53–55)	57 (55–58)
<i>st2-st2</i>	62	66	65 (61–70)	62 (60–64)	62 (60–63)	63 (61–65)
<i>st5-st5</i>	60	56	62 (58–69)	58 (56–60)	61 (60–63)	61 (58–63)
VSL	98	106	104 (95–117)	100 (99–100)	97 (90–105)	99 (98–100)
VSWant	70	72	73 (66–80)	67 (63–72)	75	70 (65–75)
VSWpost	54	56	58 (51–65)	52 (52–53)	62 (60–63)	56 (55–58)
Calyx	6	7	7 (6–9)	9 (8–10)	7 (6–9)	10 (8–11)
MDL	31	30	28 (25–30)	27 (26–27)	32 (31–33)	30
MD teeth	3	3	3	3	3	3
FDL		27	29 (26–31)	27 (26–27)	30	34 (33–35)
FD teeth	8	6	7 (5–7)	8	6	7
<i>SgeIV</i>	22	19	20 (16–24)	20 (19–21)	20 (20–21)	22 (21–22)
<i>StilV</i>	19	17	18 (15–20)	19 (16–23)		18 (17–19)
<i>StlV</i>	32	29	31 (25–36)	25 (23–29)	32 (30–33)	32 (31–32)

*Holotype; DSL: Dorsal shield length; DSW: Dorsal shield width; VSL: Ventrianal shield length; VSWant: Ventrianal shield width at level *J1/2*; VSWpost: Ventrianal shield width at level anus; MDL: Movable digit length; FDL: Fixed digit length.

j5 14 (12–17), *j6* 16 (13–21), *J2* 17 (14–21), *J5* 8 (7–9), *z2* 19 (17–22), *z4* 23 (20–26), *z5* 14 (12–18), *Z1* 20 (18–25), *Z4* 31 (30–35), *Z5* 40 (35–47), *s4* 28 (24–30), *S2* 25 (20–28), *S4* 17 (15–19), *S5* 14 (12–15), *r3* 20 (18–21), *R1* 14 (12–15). Setae *r3* and *R1* on dorsal shield. Setae *j1*, *j3*, *z2*, *z4*, *Z1*, *Z4*, *Z5*, *s4*, *S2* and *S4* serrate; *j4*, *j5*, *j6*, *z5*, *S5*, *r3* and *R1* lightly serrate and *J5* smooth.

Venter — Sternogenital shield smooth; ventrianal shield subtriangular, reticulate, 101 (92–113)

long and 134 (125–145) wide at anterior corners, with three pairs of pre-anal setae (*JV1*, *JV2* and *ZV2*), two pairs of lyrifissures and one pair of pores postero-mesad of *JV2*; *JV5* 23 (21–27). All ventral setae smooth, except *JV5* serrate.

Peritreme — Extending anteriorly to *j1*.

Spermatodactyl — Shaft L-shaped, 14 (12–16) long.

Legs — Macrosetae smooth and with tiny distal

knobs; lengths: *SgeIV* 14 (12–15), *StiIV* 14 (13–16), *StIV* 24 (21–28). Chaetotatic formula of genus II: 2-2/0-2/0-1 and of genus III: 1-2/1-2/0-1.

Material Examined — Holotype female of *N. tunus*, on *Psidium guajava* L. (Myrtaceae), October 1963, Tunapuna, Trinidad; holotype female of *N. neotunus*, on *Pothomorphe sidaefolia* (Link & Otto) Miq. (Piperaceae), March 1967, Piracicaba, São Paulo state, Brazil. Additional specimens: 1 female, on *Hevea brasiliensis* (Müll. Arg.) (Euphorbiaceae), March 2002, Nhandeara (20°42'S, 49°58'W), São Paulo, Brazil; 1 female, on *Cedrela fissilis* Vell. (Meliaceae), February 2003, São José do Rio Preto (20°46'S, 49°19'W), São Paulo, Brazil; 1 female, on *Croton floribundus* Spreng. (Euphorbiaceae), São José do Rio Preto (20°46'S, 49°19'W), São Paulo, Brazil; 1 female, on *Copaifera langsdorfii* Desf. (Leguminosae), July 2004, Itiquira (17°23'S, 54°42'W), Mato Grosso, Brazil; 2 females, on *Actinostemon communis* (Müll. Arg.) Pax. (Euphorbiaceae), July 2007, Taquaritinga (21°24'S, 48°41'W), São Paulo, Brazil; 2 females, on *A. communis*, July 2007, Barretos (20°38'S, 48°45'W), São Paulo, Brazil; 2 females and 1 male, on *A. communis*, July 2007, Turmalina (20°00'S, 50°26'W), São Paulo, Brazil; 1 female and 1 male, *A. communis*, July 2007, Matão (21°37'S, 48°32'W), São Paulo, Brazil; 1 female, on *A. communis*, July 2007, Novo Horizonte (21°03'S, 49°18'W), São Paulo, Brazil; 1 female and 1 male, on *A. communis*, April 2008, Novo Horizonte (21°03'S, 49°18'W), São Paulo, Brazil; 1 female, *A. communis*, April 2008, Palestina (20°19'S, 49°30'W), São Paulo, Brazil; 3 males, *Croton urucurana* Baill. (Euphorbiaceae), March 2008, Matão (21°37'S, 48°32'W), São Paulo, Brazil; 1 female, on *Hevea brasiliensis* (Müll. Arg.) (Euphorbiaceae), October 2008, Igrapiúna (13°48'S, 39°10'W), Bahia, Brazil; 1 female, on *Schefflera* sp. (Araliaceae), October 2008, Igrapiúna (13°48'S, 39°10'W), Bahia, Brazil; 1 female, *Pouteria torta* (Mart.) Pierre (Sapotaceae), December 2009, Chapadão do Sul (18°51'S, 52°35'W), Mato Grosso do Sul, Brazil; 1 female, *Fuirena umbellata* Rottb. (Cyperaceae), January 2010, Chapadão do Sul (18°51'S, 52°35'W), Mato Grosso do Sul, Brazil; 1 female, *Didymopanax vinosus* (Cham. & Schltdl.) Marchal (Araliaceae), December 2009, Chapadão do

Sul (18°51'S, 52°35'W), Mato Grosso do Sul, Brazil; 1 female, *Campomanesia pubescens* (Mart. ex DC.) O.Berg (Myrtaceae), December 2009, Chapadão do Sul (18°51'S, 52°35'W), Mato Grosso do Sul, Brazil; 1 female, *Miconia albicans* (Sw.) Steud. (Melastomataceae), December 2009, Chapadão do Sul (18°51'S, 52°35'W), Mato Grosso do Sul, Brazil; 1 female, *Bahuinia* sp. (Leguminosae), December 2009, Chapadão do Céu (18°15'S, 52°44'W), Goiás, Brazil; 1 female, *Stryphnodendron adstringens* (Mart.) Coville (Leguminosae), December 2009, Tupaciguara (18°31'S, 48°54'W), Minas Gerais, Brazil; 1 female, *M. albicans*, Tupaciguara (18°31'S, 48°54'W), Minas Gerais, Brazil; 1 female and 1 male, on *Myrcia palustris* DC. (Myrtaceae), April 2012, Teutônia (29°23'S, 51°48'W), Rio Grande do Sul, Brazil; 2 females, on *Myrcia palustris* DC. (Myrtaceae), April 2012, Teutônia, Rio Grande do Sul, Brazil; 2 males, on *Myrciaria plinioides* D. Legrand (Myrtaceae), April 2012, Lajeado (29°26'S, 51°57'W), Rio Grande do Sul, Brazil; 3 females and 1 male, on *Myrciaria plinioides* D. Legrand (Myrtaceae), December 2012, Lajeado, Rio Grande do Sul, Brazil; 1 female, on *Alibertia edulis* (Rich) A. Rich (Rubiaceae), February 2015, Urutaí (17°28'S, 48°12'W), Goiás, Brazil; 1 female, on *Cecropia pachystachya* Trécul. (Urticaceae), March 2015, Pires do Rio (17°25'S, 48°23'W), Goiás, Brazil; 1 female, on *Dilodendron bipinnatum* Radlk. (Sapindaceae), April 2015, Caldas Novas (17°39'S, 48°44'W), Goiás, Brazil; 2 females, on *Rudgea viburnoides* (Cham.) Benth. (Rubiaceae), May 2015, Santa Cruz de Goiás (17°20'S, 48°37'W), Goiás, Brazil; 1 female, on *Chomelia martiana* Mull. Arg. (Rubiaceae), July 2015, Orizona (16°59'S, 48°19'W), Goiás, Brazil; 2 females, on unidentified plant, April 2016, Palmelo (17°19'S, 48°24'W), Goiás, Brazil; 1 female, *Hymenaea* sp. (Leguminosae), April 2016, Palmelo (17°19'33"S, 48°24'43"W), Goiás, Brazil; 1 female, on *Piptocarpha rotundifolia* (Less.) Baker (Compositae), June 2016, Urutaí (17°24'S, 48°04'W), Goiás, Brazil.

Remarks

Denmark & Muma (1973) distinguished *Neoseiulus neotunus* from *N. tunus* based on setal ornamentation, shape of spermatheca and of leg IV

macrosetae. Our examination of both holotypes and of the additional specimens from Brazil led us to conclude that the differences mentioned in the original description of *N. neotunus* correspond to intraspecific variations. All dorsal setae, except *J5* are serrate and macrosetae of leg IV have the tips with minute knobs in both holotypes and in the additional specimens examined. The difference in the shape of the calyx of the spermatheca mentioned in the original description of *N. neotunus* might be due to the slightly different position in the mounted specimens. Additionally, the measurements include in this work and those available in the literature, for specimens from different populations are similar (Table 1). The only morphological difference observed between the holotypes was the number of teeth on fixed cheliceral digit (8 and 6 in the holotypes of *N. tunus* and *N. neotunus*, respectively). This variation has been also observed on different populations (Table 1). However, variations in number of cheliceral teeth is well known to occur in phytoseiids. In one of the extreme cases, Lofego *et al.* (2009) observed the number of teeth on the fixed digit of *Neoseiulus benjamini* (Schicha) to vary from 6 to 10 within a same population (in some specimens, between right and the left chelicerae). It is concluded that *N. neotunus* is a junior synonym of *N. tunus*.

Parthenogenesis

About 83.0% of the CAMP (25 specimens) population and 96.6% of the ICEM (29 specimens) population reached the adult stage, all being females. Emergence of the adult and pre-oviposition period were respectively 5.2 ± 0.21 and 3.9 ± 0.32 days for the CAMP (n= 24) population and 4.1 ± 0.18 and 2.7 ± 0.16 days for the ICEM (n= 26) population. Daily oviposition rates were 0.8 ± 0.06 and 1.0 ± 0.06 eggs/ female for the CAMP (n= 24) and ICEM (n= 26) populations, respectively. All of these were viable and produced only females, strongly suggesting that *N. tunus* reproduces by thelytokous parthenogenesis.

However, the occurrence of males of this species in field collected populations indicates that deutero-tokous parthenogenesis as reproductive mecha-

nism in this species should not be discarded. Reasons for the occurrence of parthenogenesis are not always known (Tilquin and Kokko, 2016). For invertebrates, including arthropods, studies have reported the modulation of parthenogenesis by abiotic and biotic factors, when this is an option for a sexual reproduction (see Tilquin and Kokko, 2016). Phytoseiids often experience various abiotic and biotic types of pressures (e.g. temperature, humidity, agrochemicals, light incidence, cannibalism, food availability, pathogens) under field and laboratory conditions, which can affect physiological and genetic mechanisms (see Ghazy *et al.*, 2016).

One of the possible biotic factors to be considered is interaction with endosymbionts, which has been reported for mites and other arthropods (Enigl and Schausberger, 2007; Zug and Hammerstein, 2012). Several arthropods have been reported infected by endosymbiotic bacteria, such as *Wolbachia* and *Cardinium* (Breeuwer and Jacobs, 1996; Kostas and O'Neill, 1998; Weeks *et al.*, 2003; Wu and Hoy, 2012). These endosymbionts have been associated with reproductive manipulation, such as cytoplasmic incompatibility, parthenogenesis and feminization (Weeks *et al.*, 2001; Kageyama *et al.*, 2012). In a study conducted by Zchori-Fein *et al.* (2001), thelytokous parthenogenesis modulated by *Cardinium hertigii* was demonstrated in wasp parasitoids of the genus *Encarsia* (Hymenoptera: Aphelinidae). Weeks and Breeuwer (2001) reported the induction of parthenogenesis by *Wolbachia* in *Bryobia praetiosa* Koch (Tetranychidae). These facts suggest that endosymbionts could also modulate the reproductive mechanism of *N. tunus*.

Under a pragmatic point of view, thelytokous reproduction may facilitate the persistence of a naturally evolved or an artificially selected characteristic (e.g. ability to diapause, tolerance to temperature variation or pesticides) in organisms released in the field for biological control purposes (Hoy and Cave, 1986). Released females would not interbreed with indigenous individuals, which could attenuate with time the predetermined characteristic.

In summary, *N. neotunus* is concluded to be a junior synonym of *N. tunus*, a species that can reproduce parthenogenetically, although in at least some

populations male specimens can be found. Complementary studies are necessary to determine factors interfering with its reproductive mechanisms and leading to the occasional production of males.

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