Fleas of the genus *Tetrapsyllus* (Siphonaptera:Rhopalopsyllidae) associated with rodents from Northwestern Argentina

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https://doi.org/10.1016/j.ijppaw.2019.03.001

Received 31 October 2018; Received in revised form 5 February 2019; Accepted 7 March 2019

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**ABSTRACT**

A new species of flea (Siphonaptera:Rhopalopsyllidae) is described from sigmodontine rodents collected during a survey of small mammals in northwestern Argentina. The new species belongs to the subgenus *Tetrapsyllus* and can be distinguished from all species of the subgenus by characteristics of the modified abdominal segments and genitalia. A phylogenetic analysis of the genus *Tetrapsyllus* using morphological characters is presented and the analysis supports the erection of this new *Tetrapsyllus* species in the subgenus *Tetrapsyllus*. The unique occurrence of a sinus in the caudal margin of the sixth sternite of females that is present only in the subgenus *Tetrapsyllus* (also unique across the Order Siphonaptera) is provided with a discussion of how this adaptation is mirrored in the parallel development of a spur on the sclerotized inner tube of males. Additional host and locality data are recorded for *Tetrapsyllus* (P.) *bleptus*. A key to the genus *Tetrapsyllus* is provided. The increased diversity of flea fauna bordering a known plague endemic area in northwestern Argentina may prove useful in implementing sustainable control measures in the future.

**Keywords:**
Tetrapsyllus
Flea
Phylogenetic analysis
Morphology
New host
Argentina

**ARTICLE INFO**

1. Introduction

Understanding the epidemiology of vector-borne diseases is strongly linked to the comprehensive the biodiversity of known or potential insect vectors, such as fleas, that may transmit pathogenic organisms to wild and domestic animals and even to humans (Bonvicino et al., 2015). In recent years, flea-borne diseases are emerging or reappearing around the world, their incidence is increasing and their distribution is changing and expanding (Bitam et al., 2010). Therefore, it is necessary to carry out studies in endemic areas of diseases transmitted by fleas, or regions that are bordering these areas, with the objective to know the flea fauna and establish a systematic basis, useful in implementing sustainable control measures in the future.

The flea family Rhopalopsyllidae includes among others, the genera *Delostichus* and *Polygenis*, which have been incriminated as vectors of *Yersinia pestis* responsible for plague in the Chile-Andean subregion (Macchiavello, 1948; Dubyanskiy and Yeszhanov, 2016). This family, represented by two subfamilies, Parapsyllinae and Rhopalopsyllinae, includes the highest number of endemic taxa in South America (Linardi and Guimarães, 2000). The taxonomy of the family Rhopalopsyllidae is quite complex due to the diversity of their morphological structures, but in spite of this, some species belonging to this family have been described recently (Hastriter and Sage, 2009, 2011; Beaucournu et al., 2013). Members of the genus *Tetrapsyllus* (subfamily Parapsyllinae) parasitize mainly small rodents of the family Cricetidae, although some species have been recorded on caviomorph rodents (Smit, 1987; Laireschi et al., 2016). The geographical distribution of known species of *Tetrapsyllus* ranges from Colombia, through Ecuador and Peru to southern Argentina and Chile (Smit, 1987). The genus is divided into three subgenera, *Tetrapsyllus* Jordan, 1931, *Heteropsyllus* Beaucournu and Torres-Mura, 1986 and *Phylliver* Smit, 1987. The subgenus *Tetrapsyllus* comprises eight species: *Tetrapsyllus coreyi* (Rothschild, 1904); *Tetrapsyllus amplus* (Jordan and Rothschild, 1923); *Tetrapsyllus tantillus* (Jordan and Rothschild, 1923); *Tetrapsyllus comis* Jordan, 1931; *Tetrapsyllus rhombus* Smit, 1955; *Tetrapsyllus contortrix* Jameson and Fulk, 1977; *Tetrapsyllus simulans* Jameson and Fulk, 1977 and *Tetrapsyllus*...
maurusinus Beaucournu and Gallardo, 1978. The subgenus Phylliver includes three species: Tetrapsyllus bleptus (Jordan and Rothschild, 1923); Tetrapsyllus tristis Johnson (1957) and Tetrapsyllus elatus Johnson (1957). The subgenus Heteropsyllus has only one species: Tetrapsyllus satyrus Beaucournu and Torres-Mura (1986). In Argentina, five of these species have been recorded: T. (H.) satyrus; T. (P.) bleptus, T. (T.) maulinus, T (T.) rhombus, and T. (T.) tantillus (Lareschi et al., 2016). The medical importance of this genus is little known, but some species such as Tetrapsyllus (T.) comis have been recorded in endemic areas of plague, e.g. Peru (MINSA, 2010).

In the present study, we describe a new species of Tetrapsyllus (Tetrapsyllus) collected in Salta province, Argentina, and include a phylogenetic analysis. Additionally, host and locality data are recorded for Tetrapsyllus (P.) bleptus. An identification key for species of Tetrapsyllus is also provided.

2. Material and methods

2.1. Flea collection

Fleas were collected from several localities in the following provinces of northwestern Argentina: Catamarca, Jujuy, La Rioja, Salta and Tucumán. The localities belong to three different eco-regions: Yungas Forests, Monte Desert of Mountains and Isolated Valleys and Puna. Some of these localities are distributed in the Andean biogeographic region (as defined by Morrone, 2015) (see Fig. 1). Specimens were collected for projects on “Diversity of Birds and Mammals of Northwestern Argentina 1998–2008” directed by one of us (RMB) and granted by CONICET PIP N 4963, and CIUNT 26/Z103 and 26/G207, with authorizations issued and supported by the provincial authorities for administration of the natural resources: Dirección de Recursos Naturales Renovables de la Provincia de Jujuy (CONST 06, WPS), Dirección Provincial de Medio Ambiente y Recursos Naturales de Salta (authorization to RMB issued June 1998) and Secretaría de Estado de Agricultura y Ganadería de la provincia de Tucumán (authorization to RMB issued June 1998). The rodents were captured with Sherman live traps baited with peanut butter and oats, and the preparation and data collection follows Díaz et al. (1998). The identification of rodents was made with keys (e.g. Díaz et al., 1997; Díaz, 2000) and by comparison with specimens deposited in collections. Mammal nomenclature follows Wilson and Reeder (2005), Barquez et al. (2006) and Patton et al. (2015). The host specimens were deposited at the Colección Mamíferos Lillo (CML), Universidad Nacional de Tucumán, Argentina, and at the Sam Noble Oklahoma Museum of Natural History (SNOMNH) (Norman, Oklahoma). Some of the host specimens are still being catalogued for the CML and hence the abbreviations used in the text correspond to the initials of the collectors: MIC (María Ines Carma) and ROTS (Rocío Tatiana Sánchez). The hosts labeled with the initials of María Ines Carma (MIC) were not available for review and so the species could not be confirmed.

The hosts were subjected to a thorough post-mortem visual examination. Fleas were removed with brushes and forceps and then preserved in 70% ethanol.

2.2. Flea examination

Fleas were prepared following conventional techniques for taxonomic identification (Hastrioter and Whiting, 2003). The images were prepared using an Olympus BX61 Compound Microscope with an Olympus CC12 digital camera in conjunction with an Olympus Microsuite B3SV program (Olympus, Melville, New York). Original descriptions of the species and the keys of Johnson (1957) and Smit (1987) were used for comparative purposes. Anatomical terms were adapted from Rothschild and Traub (1971) and the classification given by Whiting et al. (2008) was followed. Unless otherwise specified, counts of setae and spinellets represent only one side of the flea.

Landmarks used to measure the fleas are described in Hastrioter and Eckerlin (2003). For comparative purposes, specimens of Tetrapsyllus (P.) bleptus (Jordan and Rothschild, 1923) [1 female holotype (BMNH-1923-615), 1 female paratype (BMNH-1923-615), 1 male non-type (BMNH-1959-683)] from Argentina, Tetrapsyllus (T.) comis [one female holotype (BMNH-1923-615)] from Ecuador, Tetrapsyllus (T.) contortrix [1 male holotype (BMNH-1978-436)] from Chile, and Tetrapsyllus (T.) contortrix [1 male holotype (BMNH-1923-615), 1 male holotype (BMNH-1923-615), 1 male holotype (BMNH-1923-615)] from Chile, Tetrapsyllus (T.) simulans [1 male holotype (BMNH-1978-436)] from Chile, Tetrapsyllus (T.) tantillus [1 female holotype (BMNH-1923-615), 1 male non-type (BMNH-1962-698)] from Argentina, and Tetrapsyllus (P.) tristis [1 male paratype (BMNH-1957-239), 1 female paratype (BMNH-1957-239)] from Peru, were examined. These specimens are deposited in the Natural History Museum, London, U.K. Other species examined were Tetrapsyllus (T.) maulinus [1 male non-type (MGH), 1 female non-type (MGH)] and Tetrapsyllus (H.) comis [1 male non-type (MGH), 1 female non-type (MGH)] from Chile. These specimens are stored in the flea collection of J. C. Beaucournu and are being catalogued in the Museum of Natural History, Paris, France; the initials designated as MGH (Milton H. Gallardo) correspond to the collector. The holotype, allotype and paratypes of the new species of Tetrapsyllus were deposited in the Colección Mamíferos Lillo ‘Anexos’ (CMLA), Universidad Nacional de Tucumán, Argentina.

2.3. Phylogenetic analysis

In order to determine the phylogenetic placement of the new species, 24 characters based on morphology were scored for all known Tetrapsyllus species (Appendix 1). A phylogenetic tree was generated using maximum parsimony (MP) implemented by TNT (Tree analysis using New Technology) (Goloboff et al., 2003). A strict consensus tree was generated from the two most parsimonious trees found. Tree searches were conducted using the heuristic search algorithm of TNT option with 100 replications and tree bisection and reconnection (TBR) branch swapping. Multistate characters were treated as non-additive, and characters not observed were coded as” ?” (Table 1). Estimates of nodal support for the obtained trees were computed by Symmetric Resampling performed to estimate group support on unweighted results with 10,000 iterations and a probability of character weight change (up or down) of 33%. For the outgroups Polygenis (P.) acodontis, Delostichus talis and Ectinorus (E.) hapalus, currently placed in the same family, were chosen.

3. Results

Tetrapsyllus (Phylliver) bleptus (Jordan and Rothschild, 1923).

3.1. Taxonomic summary

Type host and locality. Reithrodon auritus (Fisher, 1814); Otro Cerro, Catamarca, Argentina (originally cited as Reithrodon caurinus Thomas, 1920).

Other known hosts. Abrocomidae: Abrocoma sp.; Cricetidae: Abrothrix jelskii (Thomas, 1894) [originally cited as Chrooemys pulcherrimus (Thomas, 1897)]; Abrothrix sp.; Akodon albiventer Thomas, 1897; Akodon sp.; Calomys lepidus (Thomas, 1884); Gramospis griseoflavus (Waterhouse, 1837); Phyllotis andium (Thomas, 1912); Phyllotis darwini (Waterhouse, 1837); Phyllotis magister Thomas, 1912; Phyllotis xanthopus (Waterhouse, 1837); Phyllotis sp.; Chinchillula sahamae Thomas, 1898; Eligmodontia sp.; Pumolulus lemmus Osgood, 1943. Ctenomyidae: Ctenomys jurus Thomas, 1920 (originally cited as C. medocinus jurus); Ctenomys opimus Wagner, 1884; Ctenomys sp. (Smit, 1987; Hastrioter et al., 2002; Beaucournu et al., 2014; Lareshi et al., 2016).

Known geographical distribution. Argentina: Catamarca, Jujuy, Mendoza and Neuquén provinces; Chile: Iquique and Partinacota provinces; Peru: Ancash, Junín and Puno departments (Johnson, 1957;
Material examined. CATAMARCA: La Juntas (28°06′00.00″S, 65°55′00.00″W, 1750 m a.s.l.), VI.2005, 2 ♀ (CMLA 800, 801), VII.2005, 1 ♂ (CMLA 802) from *Akodon spegazzinii* Thomas, 1897 (MIC 128, 173); Paycuqui (25°54′53″S, 67°21′15″W, 3664 ± 22 m a.s.l.), 27. III.2001, 3 ♀ (CMLA 803, 804, 805) from *Abrothrix andina* (Philippi, 1858) (CML 9218), 28. III.2001, 1 ♀ (CMLA 806) from *Neotomys ebrarius* Thomas, 1894 (SNOMNH 33874), 1 ♀ (CMLA 807) from *A. andina* (CML 6878). JUJUY: Quebrada Seca, Alfarcito, 10 km al O de Tilcara (23°34′40.53″S, 65°29′29.58″W, 3558 m a.s.l.), 13. IV.2015, 1 ♀ (CMLA 808) from *P. xanthopygus* (CML 12308). LA RIOJA: Quebrada de Santo Domingo 30 km al SO de Jagué (28°31′34.7″S, 68°46′13.8″W, 3131 m a.s.l.), 5. III.2005, 1 ♀ (CMLA 809) from *P. xanthopygus* (RTS 208); Puesto Tres Piedras (28°50′39.8″S, 67°46′58.7″W, 3304 m a.s.l.), 26.1.2016, 2 ♀ (CMLA 810, 811) from *Akodon sp.* (RTS 287). SALTA: Finca Barba Yaco, 8.5 km SE Ojo de Agua (25°58′36.8″S, 64°55′36.9″W, 1347 ± 57 m a.s.l.), 1. IX.1998, 1 ♀ (CMLA 812) from *Akodon simulator* Thomas, 1916 (SNOMNH 29996). TUCUMAN: Cienaga Escondida (26°53′20.06″S, 65°49′48.17″W, 3940 m a.s.l.), 6. XII.2016, 1 ♀ (CMLA 816) from *Phyllotis osilae* J. A. Allen, 1901 (released); Los Chorrillos 13 km NW, northern limit of Estancia Los Chorrillos (26°16′10.96″S, 65°0′53.31″W, 1592 m a.s.l.).

3.2. Remarks

In Argentina, *Tetrapsyllus (P.) bleptus* was previously reported from Catamarca, Jujuy, Mendoza and Neuquén provinces (Lareschi et al., 2016). The present finding represents the first record of the species for La Rioja, Tucuman and Salta provinces, and new localities from Jujuy.
and Catamarca provinces are added. The rodents, *A. simulator*, *A. spegazzinii* and *P. osilae* represent new hosts for this flea.

*Tetrapsyllus* (T. spegazzinii) n. sp. López Berrizbeitia, Hastirter, Barquez y Díaz.

3.3. Type material

Holotype ♂ (CMLA 818), ex *A. spegazzinii* (SNOMNH 33007), ~15 km W Escoipe, on Provincial road No. 33, Salta Province, Argentina (25°10′25.2″S; 65°49′31.6″W, 2680 m a.s.l.), 15.V.1999; allopolyte ♀ (CMLA 819): same locality data as holotype.

Paratypes: ~15 km W Escoipe, on Provincial road No. 33, Salta Province, *A. spegazzinii* (SNOMNH 33007), 65°49′31.6″W, 2680 m a.s.l., 15.V.1999; allopolyte ♀ (CMLA 819): same locality data as holotype.

3.4. Diagnosis

The new species can be distinguished from all other species of the genus by characters of the modified abdominal segments and genitalia. The male of *Tetrapsyllus (T.) spegazzinii* n. sp. differs from other species by the following combination of characters: (a) manubrium of clasper short and narrowing towards the apex (Fig. 2e), (b) telomere with several minute setae on mesal surface, and acute apex extending beyond the apex of the basimere, (c) distal arm of sternite IX (S-IX) long and width uniform along the structure (Fig. 2d,e), (d) sclerotized inner tube with a ventral triangle-shaped lobe. In the female, the shape of spermatheca is unique as the projection of cribriform area is oriented perpendicularly to the insertion of the bulga (Fig. 3b).

3.5. Description

Head (Fig. 2a and b). Front of head with prominent and massive tubercle extending to oral angle. Eyes well developed. Row of eight setae anterior to eye; one long seta below eye. Setae of second segment (pedicellus) of antenna reaching to middle of clava. Small setalae (22–27 ♀; 10–12 ♂) bordering dorsal margin of antennal fossa. Ventral margin of genal process with row of 11 setae. Labial palpus with four segments (excluding palp bearing maxillary segment) not reaching apex of fore coxa. In both sexes, occipital area with one row of seven setae plus intercalaries and one large seta anterior to this row.

Thorax. Pronotum with one row of 11 or 12 setae plus intercalaries. Mesonotum with three rows of setae, first and second rows very close, and collar with row of eight pseudosetae. Metanotum with two rows of setae, first row with 10–14 setae and second row with eight setae plus intercalaries. Mesosternum with one long seta and 2–3 small setae. Meseptemeron with single seta. Lateral metanotal area with 1–2 setae. Pleural arch and ridge well developed. Metepisternum with two setae at posterior margin. Metepisternum with two rows of setae, anterior with 7–8 setae and posterior with 5–6.

Legs: Fore-coxa with scattered setae over entire surface. Meso- and hind-coxa with small scattered setae mainly at anterior margin. Oblique break-line of meso-coxa complete. Fore-, meso- and hind femoro-tibial joints with one short lateral seta and one long mesal seta. Lateral surface of fore- and meso-femur with two rows of setae. Lateral surface of hind femur with two rows of setae: subventrally with 10 setae and dorsally with 1–5 setae. Mesal surface of hind femur coarsely striated. Margin of fore-, meso- and hind tibia with six dorsal notches. Lateral surface of hind tibia with eight or nine dorsolateral setae and seven or eight small ventrolateral setae. Mesal surface of hind tibia spiculose, as in *T. contortrix*, *T. simulans*, *T. rhombus* and *T. amplus*.

Unmodified abdominal segments: Tergites I–V with 12–17, 8–11, 5–5, 5–8, 1–5 apical setae in both sexes (Fig. 2c). Tergites I–VII with two rows of setae, anterior with short setae and posterior with long setae plus intercalaries. Tergite VII (T-VII) with two long antensal bristles. Sensillum with 15–17 sensillar pits. Stermites III–VII with a main row of four setae.

Modified abdominal segments of male (Fig. 2d and e): Posterior margin of sternite VIII with seven long setae. Basimere noticeably narrow apically, ventro-posterior margin convex with thin setae below and thicker setae above. Mesal surface of basimere with several minute setae and 2–3 long setae on apical margin. Manubrium short and narrowing towards apex. Telomere (movable process) with several minute setae on mesal surface and acute apex extended forward beyond apex of basimere. Proximal arm of S-IX similar to other species of genus, straight and unmodified. Shape of DA9 (discal arm of ninth sternum) similar to *T. (T.) corfidi*, but in new species it is longer and uniform in width along structure. Apex of DA9 rounded, apex and anterior margin with group of minute setae, and posterior margin with 3–5 small setae. Aedeagus: Distalolateral lobe expanded anteriorly with rounded apex covering median dorsal lobe. Median dorsal lobe with apical sclerite well developed. Sclerotized inner tube slightly curved upwardly apically with ventral triangular lobe. Dorsal armature well developed. Crochet hook-shaped with thick sclerotization on anterior margin flaking inner tube. Fulcrum heavily sclerotized, fulcral latero-ventral lobe narrow and long, fulcral medial lobe short and broad. Crescent sclerite thin and short, almost as long as fulcral medial lobe. Aedeagal apodeme broadly spatulate, rounded apically. Penis rods not coiled, extending slightly beyond apex of aedeagal apodeme.

Modified abdominal segments of female. Spiracle on T-VIII with two rows of setae above (seven anterior, five posterior) and two rows below
(10 setae each); numerous scattered setae behind latter row. Anal stylet short, length twice its width, with long apical seta about four times length of stylet. Sternite VI with lateral deep sinus (Fig. 3b). Posterior margin of S-VII with small lobe on ventral margin. Spermatheca similar to that of T. (T.) tantillus and T. (T.) comis, hilla longer than bulga and strongly curved. Cribriform area forming projection perpendicular to long axis of bulga, whereas the projected cribriform area in T. (T.) tantillus is parallel at its insertion on bulga (Fig. 3b).

Fig. 2. *Tetrapsyllus* (*Tetrapsyllus*) spegazzinii n. sp. (CMLA 818, 819) (a) Head, male holotype. Scale = 100 μm. (b) Head, female allotype. Scale = 200 μm. (c) Abdominal tergites, female allotype. Scale = 200 μm. (d) Basimere (BAS) and telomere (TEL), male holotype. Scale = 200 μm. (e) Terminalia. Manubrium (MAN), distal arm of sternite IX (DA9), sclerotized inner tube (SIT), male holotype. Scale = 200 μm.

Fig. 3. Female spermatheca. (a) *Tetrapsyllus* (*Heteropsyllus*) satyrus (MGH), hilla (HI) angled (white arrow). Scale = 100 μm. (b) *Tetrapsyllus* (*Tetrapsyllus*) spegazzinii n. sp. allotype (CMLA 819), hilla (HI) not angled (white arrow), bulga (BU), cribriform area (CRIA) Scale = 200 μm.
3.6. Etymology

*Tetrapsyllus spegazzinii* is named for the host *Akodon spegazzinii* from which it was collected.

3.7. Data analysis

Obtained two most parsimonious trees (Fig. 4a and b) and was generated a strict consensus tree (Fig. 5) with 57 steps. The cladogram of strict consensus consists of two major lineages. One includes *T. elutus* and the sister species *T. tristis* and *T. bleptus*. This clade is supported by character 5. These three species belong to the subgenus *Phylliver*. The other major clade, supported by character 12, includes a polytomy conformed by *T. contortrix*, *T. comis*, *T. tantillus* and *T. satyrus* and two clades, one supported by character 15 and comprises *Tetrapsyllus spegazzinii* n. sp. + *T. corfidii* and other supported by characters 11 and 20 and includes *T. rhombus* and the sister species *T. amplus*, *T. simulans* and *T. maulinus*.

*Tetrapsyllus satyrus* is the unique species of the subgenus *Heteropsyllus* but its placement is better represented in tree 1, where it appears as a sister species of the species belonging to the subgenus *Tetrapsyllus* (Fig. 4a).

In the three cladograms, most of the clades showed low support values (Fig. 6a and b, 7).

4. Discussion

Previously only one species belonging to the genus *Tetrapsyllus*, subgenus *Phylliver*, had been recorded in the study area. The present study describes a new species of *Tetrapsyllus* using morphological characters and adds a new species for Argentina, increasing the diversity of flea fauna in northwestern Argentina. In Argentina, *Tetrapsyllus* had been represented by five species, one belonging to the subgenus *Heteropsyllus*, one to *Phylliver* and three to *Tetrapsyllus*, all parasitizing rodents of the families Cricetidae, Ctenomyidae and Octodontidae (Lareschi et al., 2016). Our study provides the first record of the subgenus *Tetrapsyllus* in northwestern Argentina and increases the number of species in the subgenus to four.

The phylogenetic analysis supports a close relationship between *Ectinorus* and *Tetrapsyllus* (Fig. 5). The recorded synapomorphies are the labial palp not extending to the apex of the procoxa (17:0), one large ventral seta anterior to the occipital row of setae (18:0), and the dorsal margin of median dorsal lobe aedeagal evenly convex (23:0); although, some of these characters are represented by gaps and polymorphisms that did not resolve. In addition, *Tetrapsyllus* is monophyletic, supported by two relevant synapomorphies: the labial palp with four segments (21:0) and a thick-walled capsule that contains the frontal tubercle (22:1).

*Tetrapsyllus* (T.) *spegazzinii* n. sp. is the sister species of *T. corfidii* in the three trees (Fig. 4a and b, 5). This is supported by a combination of characters shared by males and females of both species as follows: the presence of two rows of setae on the lateral surface of the hind femur (15:1), the oblique break-line of the meso-coxa complete (10:1), among other characters. The presence of a cribriform area parallel to the insertion of the bulga with the hilla in *Tetrapsyllus* (T.) *spegazzinii* n. sp. (character 12:1) is autapomorphic for this species (Fig. 4a and b). With respect to the politomies represented in the strict consensus tree (Fig. 5) and low support values in the most cladades (Fig. 6a and b, 7) we consider that additional morphological and non-morphological characters (molecular, ecological) are required in order to obtain better resolution.

*Tetrapsyllus* (T.) *spegazzinii* n. sp. belongs to the subgenus *Tetrapsyllus* which share the general appearance of male DA9 and some structures of aedeagus, and in the female in the features of sternites VI.
and VII and the shape of the spermatheca (Smit, 1987). The subgenus *Tetrapsyllus* is unique in the Order Siphonaptera in that females have the sinus in the caudal margin of S-VI instead of S-VII (Smit, 1987). We compared this character in each of the three subgenera and found a ventral triangular lobe or spur in the sclerotized inner tube of the aedeagus of males that could be complementary to the location of the sinus in the sternites of the female. In species of the subgenus *Phylliver* the ventral spur is on the basal half of the sclerotized inner tube, whereas in the subgenus *Tetrapsyllus*, it is located on the apical half. Possibly a shifting in the position of the sinus to S-VI facilitates the success of reaching the female sternite during copulation. Likewise, reports of new flea–rodent associations are important because rodents are the main hosts of fleas (Bonvino et al., 2015), accommodating 74% of known flea species (Krasnov et al., 2004).

The ecological aspects of the species analyzed in this study are still unknown. Therefore, more studies comprising a larger sample size are required to better understand the prevalence and others quantitative descriptors of species of *Tetrapsyllus* on rodents populations.

Identification key to species of *Tetrapsyllus* modified from Smit (1987).

|   |   |
|---|---|
| 1 | Male: Prominent ventral spur on sclerotized inner tube of aedeagus; female: hilla angled |
| 2 | Tetrapsyllus (Heteropsyllus) satyrus |
| 1′ | Male: ventral spur or triangular lobe on sclerotized inner tube present but not prominent; female: hilla not angled (Fig. 3a and b) |
| 3 | Tetrapsyllus (Phylliver) |
| 2′ | Male: ventral spur on the apical half of the sclerotized inner tube; female: sinus present on posterior margin of S-VI |
| 4 | Tetrapsyllus (Tetrapsyllus) |
| 3′ | Male: telomere extending beyond apex of basimere |
| 5 | T. (P.) bleptus |
| 4′ | Male: apex of telomere not extending beyond apex of basimere |
| 6 | Oblique break-line of meso-coxa interrupted medially |
| 5′ | Oblique break-line of meso-coxa complete |
| 6′ | Male: telomere broad with numerous minute setae on mesal surface; female: spermatheca with bulga pyriform |
|    | T. (T.) tantillus |
6’ - Male: telomere narrow with few minute setae on mesal surface; female: spermatheca with bulga spherical T. (T.) comis

7 - Eyes vestigial T. (T.) maulinus

7’ - Eyes well developed 8

8 - Hind femur densely striated T. (T.) confidi

8’ - Hind femur coarsely striated 9

9 - Two rows of setae on the lateral surface of hind femur T. (T.) spegazzinii sp. n.

9’ - One row of setae on the lateral surface of hind femur 10

10 - Male: small triangular ventral lobe on sclerotized inner tube of aedeagus; female unknown T. (T.) contortrix

10’ - Male: large triangular ventral lobe in the sclerotized inner tube; spermatheca with hila almost as long as bulga, female unknown for T. simulans 11

11 - Male: total length of telomere five times its width; female unknown T. (T.) simulans

11’ - Male: total length of telomere three or four times its width; female: hilla almost as long as the bulga T. (T.) rhombus

12 - Male: dorsal margin of median dorsal lobe of aedeagus evenly convex; female: occipital area with one row of setae T. (T.) amplus

12’ - Male: dorsal margin of median dorsal lobe of aedeagus apically concave; female: occipital area with two row of setae

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors thank members of the Programa de Investigaciones de Biodiversidad Argentina (PIDBA) for their help during the entirety of this study and are especially grateful to Julieta Perez for her help in the phylogenetic analysis and Tatiana Sánchez for the collection of some specimens. The authors are grateful to Professor Jean-Claude Beaucournu (Rennes, France) for the loan of specimens, and Erica McAlister, curator at the British Museum of Natural History, London, for her support during a visit by MFL-B to the museum. Field trips were funded by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. We want to thank the anonymous reviewers for their valuable comments and suggestions.

Appendix I

Morphological characters used in the matrix (Table 1).

0) Metanotum: (0) spinelets present; (1) spinelets absent.

The spinelets are small spines or modified setae (Rothschild and Traub, 1971). Species in the subfamily Rhopalopsyllinae have spinelets on the metanotum, whereas members of the family Parapsyllinae lack spinelets on the metanotum.

1) Antennal clava: (0) symmetrical; (1) asymmetrical.

The antennal clava is the third appendage of the antenna and consists of nine segments. The shape of this is structure shows great variation in different species of fleas (Snodgrass, 1946). A symmetrical antennal clava is present in species of the subfamily Parapsyllinae and asymmetrical in members of the subfamily Rhopalopsyllinae.

2) Antero-ventral projection of metasternum: (0) prominent; (1) less prominent.

Smit (1987) defined a metasternum anteriorly expanded markedly downwards forming an antero-ventral projection for species in the genera Delostichus and Tetrapysillus, whereas that, for the remaining species of the subfamily Parapsyllinae, the shape of the metasternum is different, although a small antero-ventral projection may be present.

3) Antennal pedicel1us: (0) setae absent; (1) setae present on lateral margin; (2) setae present on the posterior margin. The antennal pedicel is the second appendage of the antenna and supports the antennal clava (Snodgrass, 1946). Species of the genus Tetrapysillus have an antennal pedicel with lateral setae, as well as, the out-group Ectinorus halapaeus, whereas Delostichus talis lacks setae on the pedicel. On the other hand, Polygenis acodontis (subfamily Rhopalopsyllinae) bears setae on the posterior margin of the pedicel.

4) Sclerotized inner tube of aedeagus: (0) prominent ventral spur present; (1) small ventral spur or triangular lobe present; (2) spur or lobe absent. The sclerotized inner tube continues from the endophallus and varies in shape and length in different species of fleas (Snodgrass, 1946; Rothschild and Traub, 1971). Smit (1987) defined the presence of a structure associated with inner tube represented by a ventral spur or triangular lobe for species in the genus Tetrapysillus. Species of the subgenus Phylliver have a ventral spur, whereas the subgenus Tetrapysillus has a ventral triangular lobe. In Tetrapysillus (H.) satyrus, this structure is hyperdeveloped as a spur (Beaucournu and Torres-Mura, 1986). This structure is absent in species of the out-group. The structure probably has an important function during copulation in species of Tetrapysillus and is complemented by the position of the sinus in sternites VI or VII in the females (see discussion).

5) Sclerotized inner tube of aedeagus: (0) undulating; (1) straight or up turned apically; (2) coiled. Species of the subgenus Phylliver are characterized by having an inner tube undulating, whereas, in species of the subgenus Heteropsyllus and Tetrapysillus it is straight or upturned apically, as in two species of the out-group, D. talis and E. halapaeus. The inner tube in Polygenis acodontis is coiled with some revolutions.

6) Hilla (0) angled apically; (1) rounded apically. The spermatheca contains the bulga and the hilla with a narrow circular orifice that connects these two structures (Rothschild and Traub, 1971). Both structures vary in shape and length in different species of fleas. The hilla angled apically is present only in T. satyrus.

7) In females: (0) posterior margin of S-VII with sinus; (1) posterior margin of S-VI with sinus; (2) posterior margin of sternites VI and VII without sinus. S-VII and its posterior margin are taxonomically important in the order Siphonaptera (Beaucournu and Gomez-Lopez, 2015); however, S-VI is especially important in the genus Tetrapysillus. In the species of the subgenus Phylliver, the sinus is present on the posterior margin of S-VII, whereas, in species of the subgenus Tetrapysillus, it is located on the posterior margin of S-VI. Species in the out-group lack a sinus on the posterior margin of S-VI and S-VII.

8) Setae on the lateral surface of the hind tibia: (0) with more than 30; (1) with fewer than 20. On the lateral surface of the hind tibia, most species included in the phylogenetic analysis have scarce setae; however, T. satyrus, T. tristis, T. maulinus and one species in the out-group, P. acodontis, have numerous setae. This character was not observed in T. simulans, T. rhombus and T. amplus.

9) Apex of telomere: (0) extending beyond apex of basimere; (1) not extending beyond apex of basimere. The telomere is part of the genitalia of the male. This structure, together with the basimere and other structures, constitutes the clasper, the external genital organ (Snodgrass, 1946; Rothschild and Traub, 1971). The species of the out-group present an apex of the telomere not extending beyond the apex of the basimere, as in T. satyrus, T. elutus and T. tantillus. The remaining species of Tetrapysillus present an apex of the telomere extending beyond apex of basimere.

10) Oblique break-line of meso-coxa: (0) interrupted in the middle; (1) complete. Species of the out-group have an oblique break-line.
interrupted in the middle of the meso-coxa, as in T. bleptus, T. comis and T. tantillus, whereas the oblique break-line in the remaining species of the subgenus Tetrapsyllus is complete. This character was not observed in T. satyrus, T. tristis and T. elutus.

11) Mesal surface of telomere: (0) with scarce minute setae; (1) with numerous minute setae. The mesal surface of the telomere with scarce minute setae was recorded in most examined species, except for T. tantillus, T. maulinus, T. similans, T. rhombus, T. amplus and T. spazzanzii n. sp., species of the subgenus Tetrapsyllus, and E. hapalus.

12) Cribiform area of bulga: (0) extended perpendicular to the insertion of the bulga; (1) extended parallel to the insertion of the bulga; (2) not extended. The cribiform area is composed of the openings of the ducts of numerous individual spermathecal glands (Rothschild and Traub, 1971). Species in out-group have a cribiform area that is not extended, as do species of Tetrapsyllus belonging to the subgenus Phyliliver, whereas in the remaining species of Tetrapsyllus, the cribiform area is extended as described above (Fig. 3b).

13) Eyes: (0) vestigial; (1) well developed. Most species have well developed eyes. Tetrapsyllus maulinus is the unique species with vestigial eyes. This character was not observed in T. elutus.

14) Hind femur: (0) densely striated; (1) coarsely striated; (2) not striated. The hind femur in most species is coarsely striated; only in T. tristis and T. cordifidi are dense striations present. On the other hand, no striations are present on the hind femur of E. hapalus. This character was not observed in T. satyrus, T. tantillus, T. comis and T. maulinus.

15) Lateral surface of hind femur: (0) with one row of setae; (1) with two rows of setae; (2) with setae irregularly placed. In T. bleptus, T. tantillus, T. comis, T. contortrix, T. similans, T. rhombus, T. amplus and E. hapalus, one row of setae is present on the lateral side of the hind femur. Irregularly placed setae are present on the lateral side of the hind femur only in T. satyrus and T. tristis. The remaining species of the genus Tetrapsyllus have two rows of setae on the lateral aspect of the hind femur. In Polygenis acodondis, the three states were present; therefore, for this species the character is polymorphic.

16) Mesal surface of hind tibia: (0) spiculose; (1) not spiculose. The mesal surface of the hind tibia is not spiculose in species of the out-group and in T. cordifidi, whereas in T. bleptus and some species of the subgenus Tetrapsyllus, it is spiculose. This character was not observed in T. satyrus, T. tristis, T. elutus, T. tantillus, T. comis and T. maulinus.

17) Labial palpus to apex of procoxa: (0) not extending; (1) extending. Species of the out-group, P. acodondis and D. talis have a labial palp extending to the apex of the procoxa, as in T. bleptus, T. maulinus and T. similans; whereas in E. hapalus and the remaining species of genus Tetrapsyllus, the “0” condition is present. This character was not observed in T. rhombus and T. amplus.

18) Occipital setae: (0) one row of setae; (1) two rows of setae. Species in the subgenus Phyliliver have a large ventral seta anterior to a row of seven setae plus intercalaries, as in E. hapalus, T. tantillus, T. comis and Tetrapsyllus spazzanzii n. sp. Both states are present in Tetrapsyllus cordifidi and T. rhombus. In P. acodondis, D. talis and the remaining species of the genus Tetrapsyllus, there are more than two penultimate occipital setae. This character was not observed in T. elutus.

19) Sensillum: (0) with 16–17 sensillial pits each side; (1) with 20–21 sensillial pits each side. Most species included in the phylogenetic analysis have a sensillum with 16–17 sensillial pits on each side. Tetrapsyllus tantillus is the only species with a sensillum with 20–21 sensillial pits on each side. This character was not observed in T. elutus and T. amplus.

20) Manubrium: (0) fairly broad; (1) narrow. The manubrium is an apodeme extending from the proximal region of the basimere

(Rothschild and Traub, 1971) and it can have different shapes. The manubrium is fairly broad in some species of the subgenus Tetrapsyllus: T. tantillus, T. maulinus, T. similans, T. rhombus and T. amplus. The remaining species of the subgenus Tetrapsyllus, all species of the subgenus Phyliliver, and the out-group, have a narrow manubrium.

21) Labial palpus: (0) with four segments; (1) with five segments. This structure bears four segments in all species of the genus Tetrapsyllus.

22) Walled capsule that contains the frontal tubercle: (0) thin; (1) thick. This structure is thin only in species in the out-group.

23) Dorsal margin of median dorsal lobe of aedeagus: (0) evenly convex; (1) apically concave. The median dorsal lobe is an extension of the median apodeme of the aedeagus (copulatory organ) in the male (Rothschild and Traub, 1971). Most species included in the phylogenetic analysis have the dorsal margin of the median dorsal lobe evenly convex, except in P. acodondis, D. talis, T. maulinus, T. similans and T. amplus.
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