

A new genus of fleas with associated microorganisms in Dominican amber

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Poinar: a new fossil flea in Dominican amber

Systematics and evolution

## **Abstract**

A flea preserved in Dominican amber is described as *Atopopsyllus cionus*, n. gen., n. sp., (Atopopsyllini n. tribe, Spilopsyllinae, Pulicidae). The male specimen has two unique characters that have not been noted in previous extant or extinct fleas, thus warranting its tribal status. These characters are 5-segmented maxillary palps and cerci-like organs on abdominal T. X. Additional characters are the absence of ctenidia, very small eyes, a lanceolate terminal segment of the maxillary palps, legs with six notches on the dorsal margin of the tibiae, five pairs of lateral plantar bristles on the distitarsomeres and nearly straight ungues with a wide space between the basal lobe and tarsal claw. Trypanosomes and coccobacilli in the rectum and coccobacilli on the tip of the epipharynx of the fossil are depicted and briefly characterized.

**Keywords:** fossil flea, *Atopopsyllus cionus*, n. gen., n. sp., five-segmented maxillary palps; associated microorganisms

## **Introduction**

Fossil fleas are rare and aside from some primitive flea-like arthropods described from Cretaceous deposits, all “modern” fleas have been described in Baltic and Dominican amber (Poinar 1995). An extensive molecular phylogenetic study involving 259 flea taxa suggested that the common ancestor of all extant fleas diversified during the Cretaceous, while the majority of the intra-ordinal divergences occurred in the Paleogene (Zhu et al. 2015).

The male Dominican amber flea described here was originally identified as belonging to the extant genus *Rhopalopsyllus* Baker (Poinar 1995). However, a re-investigation showed that this flea is not a member of the family Rhopalopsyllidae and has unique features that warrant new tribal status. These properties are five-segmented maxillary palps and a pair of cerci-like organs positioned on T. X near the tip of the abdomen. A description follows, accompanied with a brief characterization of trypanosomes and coccobacilli associated with the fossil.

## Materials and methods

The fossil originated from amber mines in the northern mountain range (Cordillera Septentrional) of the Dominican Republic between Puerto Plata and Santiago. Amber from mines in this region was produced by *Hymenaea protera* Poinar (1991) (Fabaceae) and the Dominican amber forest was characterized as a tropical moist forest based on biota recovered from the amber (Poinar and Poinar 1999).

Dating of Dominican amber is controversial, with the youngest proposed age of 20-15 mya based on Foraminifera (Iturralde-Vinent and MacPhee 1996) and the oldest of 45-30 mya based on coccoliths (Cêpek in Schlee 1990). These are minimum dates since they are based on microfossils in the strata containing the amber. Most of the amber was secondarily deposited in turbiditic sandstones of the Upper Eocene to Lower Miocene Mamey Group (Draper et al. 1994). Dating is further complicated by the discovery of Early Oligocene amber in Puerto Rico and Maastrichtian-Paleocene amber in Jamaica (Iturralde-Vinent 2001) showing that amber from a range of deposits occurs in the Greater Antilles.

Observations and photographs were made with a Nikon SMZ-10 R stereoscopic microscope and Nikon Optiphot compound microscope with magnifications up to 600 X. Helicon Focus Pro X64 was used to stack photos for better clarity and depth of field.

## Results

Characters of the fossil flea that place it in the subfamily Spilopsyllinae include: dorsal abdominal segments with only a single transverse row of bristles, thorax above longer than the first abdominal segment and the absence of a frontal-epicranial suture (Ewing 1929, Brues et al. 1954, Medvedev 1998).

Order Siphonaptera

Family Pulicidae

Subfamily Spilopsyllinae

Tribe Atopopsyllini n. tribe

Diagnosis: Same as type species since monotypic.

Type genus: *Atopopsyllus* n. gen.

Diagnosis: Same as type species since monotypic.

Type species: *Atopopsyllus cionus*, n. gen., n. sp.

*Atopopsyllus cionus*, n. gen., n. sp. (Figs. 1-9)

Diagnosis. The fossil is characterized by five-segmented maxillary palps and a pair of cerci-like organs positioned near the tip of the abdomen. Additional features include the absence of ctenidia, small eyes, legs with femoro-tibial joints each bearing one short and one long guard seta, the dorsal margins of the tibiae bearing five notches for the insertion of bristles, four pairs of lateral plantar bristles and one pair of setae on the

distitarsomeres, with terminal bristles the shortest, tarsal claws almost straight, with a wide opening between the tarsal claw and the basal lobe of unguis and sensillum bordered by three tubercles.

Description. Head: Anterior margin of head without frontal tubercle, evenly rounded from its dorsal base to coxa I. Inter-antennal suture (falx) inconspicuous. No sclerotized demarcation of cephalic capsule between occipito-frontal and genal zones. Antennal fossa well developed, 282  $\mu\text{m}$  long; antennal club symmetrical, cone-shaped, with nine segments gradually decreasing in size, length, 240  $\mu\text{m}$ ; scape and pedicel half as wide as basal club segment; eye small, inserted medially between frontal margin and gena; genal apex lacking process or tooth. Maxillary palpus five-segmented; lengths of segments, I= 35  $\mu\text{m}$ , II= 46  $\mu\text{m}$ , III= 116  $\mu\text{m}$ , IV= 92  $\mu\text{m}$ , V= 127  $\mu\text{m}$ ; segment V sub-lanceolate, tapering to point (Figs. 2, 9). Labial palps and epipharynx hidden by coxa I. Chaetotaxy: three occipital rows, anterior row with 4 setae, middle row with four setae, posterior row with four setae: three occipital frontal/genal rows, anterior row with four setae, second row with three setae (including one very long ocular bristle) and third posterior row (beneath eye) with three setae.

Thorax. Pronotum not separated from head capsule, similar to mesonotum in bearing median row of 4 long stiff setae. Cervical link plate present. Metanotum dorsally equal in width to that of preceding segment, with similar chaetotaxy. Pro-, meso-, and metathoracic femora and tibiae nearly identical except for size and arrangement of femoro-tibial guard setae. Femoro-tibial joints each with one long and one short guard seta (Fig. 3). Dorsal margin of metatibia (length, 475  $\mu\text{m}$ ) with five notches, each bearing

paired stout setae; ventral margin of tibia with two stout apical setae; tarsi five-segmented with tarsomeres not notched; pro and mid tarsomeres slender, elongate with tarsomeres II and V longest and tarsomere IV shortest, less than half length of tarsomeres II and V (Fig. 4); basal tarsomeres (length with claw, 265  $\mu\text{m}$ ), with 5 pairs of ventral setae with terminal pair shorter and thicker than remainder (Fig. 4). Tarsal claws long, nearly straight, bearing coarse, minute projections basally; basal lobe third as long as claw, triangular, lacking serrations (Figs. 5,6).

Abdomen longer than high, T. I–V with row of five setae on each side; T. VII with paired ante-sensilial bristles (length, 180  $\mu\text{m}$ ) followed distally by small tubercle bearing one short spine followed by depression with very short tubercle bearing one seta. Sensillum distinct on T. VIII, followed distally by two prominent tubercles, the first bearing three clusters of fine setae; the second smaller tubercle capped by short spine and several long, fine setae. Prominent anal seta positioned posterior to these tubercles (Figs. 7-9). Pair of cerci-like organs (length, 130  $\mu\text{m}$ ) near the tip of abdomen on T. X (Figs. 8, 9). Genitalia restricted to portion of *processus basimeris* (length, 140  $\mu\text{m}$ ) protruding from base of St. VIII (Fig.7).

*Types:* Holotype male (accession # Sp-1-1) deposited in the Poinar amber collection maintained at Oregon State University, Corvallis, Oregon.

*Type locality:* Amber mine in the northern mountain ranges (Cordillera Septentrional) of the Dominican Republic. DD latitude and longitude: 19.4, -70.4.

*Etymology:* The generic name is from the Greek “atopos” = strange and the Greek “psyllo” = flea. The specific epithet is from the Greek “kion” = pillar in reference to the presence of a circus-like organ resembling an anal stylet that occurs on female fleas.

*Comments:* *Atopopsyllus cionus*, n. gen., n. sp. shares some features with the Dominican amber flea, *Eospilopsyllus kobberti* Perrichot, Beaucournu and Velten (2012), such as the number and arrangement of spines on the tibia and tarsus. However, the shape of the body clearly separates the two species, that of *E. kobberti* being essentially oval while that of *Atopopsyllus cionus*, n. gen., n. sp. is sub-rectangular. The new species also lacks the eight minute setae in a sinuous vertical row along the fronto-genal suture that are present in *E. kobberti*. *Atopopsyllus cionus*, n. gen., n. sp. also differs from both *E. kobberti* and the previously described *Pulex larimerius* Lewis & Grimaldi (1997) by its elongate protarsal segments since the protarsal segments in the latter two species are relatively short and wide.

### **Associated microorganisms**

An examination of the rectum of *Atopopsyllus cionus*, n. gen., n. sp. revealed the presence of microorganisms. One is a trypanosome with cells ranging from 34 µm to 45 µm in length. Some areas of the rectum were filled with large numbers of this protozoan (Fig. 10). Trypanosomes in fleas normally develop in the midgut, then invade and multiply in the gut cells, eventually breaking out and passing to the rectum where they are voided with the feces (Durden and Traub 2004).



Other microorganisms in the rectum are coccobacilli that are intermediate between spherical cocci and rod-shaped bacilli. They occur singly or in small groups, range from 2.0  $\mu\text{m}$  to 5.0  $\mu\text{m}$  in diameter and most are surrounded by faint opaque envelopes (Fig. 11). Attached to the tip of the epipharynx of the fossil flea is a small group of similar coccobacilli (Fig.12). These coccobacilli fall within the size range (1.0-4.0  $\mu\text{m}$ ) of vertebrate pathogenic bacteria, including *Yersinia* spp., transmitted by extant fleas (Bercovier and Mollaret 1984; Durden and Traub 2008). While it is not possible to assign these fossil cells to any known genus of bacteria, it is interesting that of the pathogenic bacteria transmitted by fleas today, *Yersinia* is the only one that forms both short rods and nearly spherical cells (Bercovier and Mollaret 1984).

### **Discussion**

The unique characters (5-segmented maxillary palps and cerci-like organs) of *Atopopsyllus cionus*, n. gen., n. sp. warrant its placement in a new tribe, the Atopopsyllini n. tribe. All extant fleas have 4- segmented palps (Smit 1957) and this condition in the fossil shows that some ancient flea lineages had 5-segmented maxillary palps. Kluge (2002) actually considered fleas as having 5-segmented maxillary palps, although his 5<sup>th</sup> segment was the modified maxillary stipe. Mecoptera, considered the sister group to fleas and scorpionflies, are characterized by 5-segmented maxillary palps (Borror et al. 1989; Beutel et al. 2008; Whiting 2002).

The paired cerci-like organs near the tip of the abdomen are difficult to interpret (Figs. 8,9). Fleas lack cerci but female fleas have structures termed anal stylets that occur

in roughly the same location as cerci in other insects. The anal stylet in fleas is an elongate structure behind the sensilium and bears one long apical seta and often one or more preapical small setae on each side of T. X (Smit 1957). In the fossil, the cerci-like organs are elongated, bifid sub-apically and bear two elongate setae on the longer tip and a single seta on the shorter one. It is difficult to determine if this cercus-like organ corresponds to the female anal stylet. Similar structures could not be found on any extant or extinct male Siphonaptera (Smit 1957; Tipton and Méndez 1966; Tipton and Machado-Allison 1972; Lewis 1998; Lewis and Lewis 1985; Medvedev 1998).

The region of the sensilium on T. X contains additional diagnostic characters of *Atoposyllus cionus*, n. gen., n. sp. including tubercles in front of and behind the sensilium. While the sensilium is usually confined to a “pocket” on the dorsum of fleas, in the fossil, sensilium-like elements also occur in depressions anterior to the sensilium (Fig. 9, arrows). These two depressions contain hair-like elements that probably served a tactile function.

Since extant spilopsylliid fleas parasitize mammals, this group of vertebrates probably also served as hosts to *Atoposyllus cionus*, n. gen., n. sp. The long, nearly straight claws with minute projections must have been adapted for a specific group of hosts. Several mammalian groups that occurred in the Dominican amber forest based on hair and bone remains found in amber include Hutia rodents (Capromyidae), Solenodon insectivores, felines and bats (Poinar and Poinar 1999; Poinar and Brown 2012).

A number of pathogenic trypanosomes occur in fleas and some are transmitted to rodents while others are thought to infect birds, shrews, voles and rabbits (Durden and Traub 2002). It is impossible to determine if the trypanosomes or coccobacilli observed

in the rectum or the coccobacilli on the epipharynx of *Atopopsyllus cionus*, n. gen., n. sp. are symbionts or vertebrate parasites that were vectored by the fossil flea.

### **Acknowledgments**

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### Figure legends

Fig. 1. Lateral view of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 465  $\mu\text{m}$ .

Fig. 2. Left maxillary palp of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 65  $\mu\text{m}$ . Upper arrow shows extra (5<sup>th</sup>) segment. Lower arrow shows tip of ocular seta.

Fig. 3. Metatibia of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 70  $\mu\text{m}$ .

Fig. 4. Protarsus of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 83  $\mu\text{m}$ . Arrow shows reduced 4<sup>th</sup> segment.

Fig. 5. Lateral view of mesotarsal claws of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 32  $\mu\text{m}$ .

Fig. 6. Ventral view of mesotarsus of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 50  $\mu\text{m}$ .

Fig. 7. Lateral view of terminal abdominal segments of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 20  $\mu\text{m}$ . A = antesensilial bristles; B = short tubercle bearing spine; C = sensillum; D = large post-sensillum tubercle; E =

small post-sensillum tubercle; F= anal seta; G = *processus basimeris*.

Fig. 8. Portion of abdominal T. IX and X of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 14  $\mu\text{m}$ . S = sensillum; T= large post-sensillum tubercle; C = cercus-like organ.

Fig. 9. *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. A. Maxillary palp. Scale bar = 76  $\mu\text{m}$ . B. Portion of abdominal T. IX and X with sensillum (S), cercus-like organ (C) and additional areas of sensory setae (B). Scale bar = 65  $\mu\text{m}$ .

Fig. 10. A cluster of trypanosomes in the rectum of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 56  $\mu\text{m}$ .

Fig. 11. Clusters of coccobacilli (arrows) in the rectum of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 7  $\mu\text{m}$ .

Fig. 12. A group of coccobacilli (arrow) adhering to the tip of the epipharynx of *Atopopsyllus cionus*, n. gen., n. sp. in Dominican amber. Scale bar = 35  $\mu\text{m}$ .