HOW DOES A BLOOD FEEDING LOUSE (PHTHIRAPTERA: INSECTA) SURVIVE STARVATION: FOUR CASE STUDIES OF LICE ON HUMMINGBIRDS (TROCHILIDAE: AVES) AND CONSEQUENCES OF OVERCROWDING OF ADULTS AND THEIR EGGS

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ABSTRACT Lice (Phthiraptera) are ectoparasites of birds and little is known about their biology. Lice of hummingbirds, studied here belong to the Ricinidae Family and feed on the host's blood. Here, we present four experimental cases to test how they cope with the lack of their blood meal, and what happens to their eggs when there is a large population in one host.

Keywords: Phthiraptera, Insecta, Lice, Eggs, Overcrowding.

RESUMO Os malófagos (Phthiraptera) são ectoparasitos de aves e pouco se conhece sobre sua biologia. Os malófagos de beija-flores estudados aqui, são da família Ricinidae, e alimentam-se de sangue de seu hospedeiro. Aqui, apresentamos quatro experimentos para investigar como eles se comportam na falta de seu alimento usual, e os eventos que ocorrem com seus ovos no caso de haver uma superpopulação em um hospedeiro.

Palavras-chave: Phthiraptera, Insecta, Malófagos, Ovos, Superpopulação.

INTRODUCTION

Phthiraptera (Order Psocodea) are flat bodied ectoparasites, mainly of birds; but mammals are known to harbor some species as well (PRICE, 1987). It is an order not well studied due to lack of field students and also due to difficulties involving the acquisition of material. Thus, the information in the literature, especially for Brazilian birds, concerns mostly, with few exceptions, in the description of new genus and species as its alpha taxonomy is still in a blooming stage, and little is known about their biology (ONIKI, 1995; VALIM, 2006, 2013; VALIM; CICCHINO, 2015a, 2015b). Also, we find in the literature, a fair amount of biological information on lice of domestic mammals and birds (TYAGI *et al.,* 2009; SAXENA *et al*., 2012); reports on occurrences (RODA; FARIAS, 1999; LYRA-NEVES *et al*., 2000, 2005).

Pthiraptera that infest hummingbirds (Trochilidae) are insects of the Order Psocodea, Superfamily Amblycera, harboring four genera: *Trochiloecetes*, *Trochiliphagus* (Family Ricinidae), *Leremenopon*, and *Myrsidea* (Family Menoponidae) (PRICE *et al.*, 2003, DALGLEISH e PRICE, 2003a, 2003b), and all feed on blood. According to CLAY (1949) *Trochiloecetes* spp. and *Trochiliphagus* spp. present modifications in the mandibles to puncture the host's skin, and feed on the host´s blood. *Trochiloecetes* spp. feed mainly on blood, as it is seen by the blood cells packed in their digestive tract; *Trochiliphagus* spp. probably feeds on blood as well but we did not see blood cells in their digestive tract; they probably feed more on body fluids and other debris in the host's body. Many of the species of both genera, parasitizing Neotropical hummingbirds are not described yet.

Species of the genus *Trochiloecetes* are stocky, and short-legged lice due to their more sedentary habits than *Trochiliphagus* sp. (ONIKI, 1999b). Representatives of this last cited lice are long-legged and run very fast throught the feathers of the host´s body, so it is considered by some authors as rare, because they are more difficult to see or to catch (pers. obs.). Both genera are represented by relatively large species, when one considers the size of a hummingbird.

Trochiloecetes spp. are very often found attached to the base of the ventral side of the rachis of a feather and next to a good egg, *i. e*., an egg recently laid or close to an ecloded egg (empty). The species usually lays its eggs on the back of the neck (nape) of the hummingbird, and slowly spread to the sides if in large number. However, they also clusters two

or even 3-4 eggs on a feather when population is high (pers. obs.)

Cicchino and Abrahamovich (1987) not only described a new species *Trochiloecetes aureoventridis* from *Chlorostilbon lucidus* (Trochilidae) but made drawings and took pictures of their eggs, showing teeth like structures that help open the eggs during eclosion; they called it the "hatching organ". The Glittering-bellied Emerald, former *Chlorostilbon aureoventris* is presently placed in *C. lucidus* (SHAW, 1812). (Apodiformes, Trochilidae) by Piacentini *et al.* (2015).

The eggshells of Phthiraptera present special markings which according to Balter (1968 a, b) are often species specific, and more distinct in the amblyceran than in ischnoceran lice.

The present work reports on experiments we did after one of those field trips to verify survival and feeding habits. We also looked at the egg condition when there is an overcrowding in the adult population.

MATERIAL AND METHODS

In 1989 (ONIKI, 1994), we put together a project to band hummingbirds in the Atlantic Forest in southeastern Brazil. The main banding site was the Museu de Biologia Mello Leitão in Santa Teresa, in Espírito Santo State, but we visited several surrounding areas, as well as surrounding states, such as, Minas Gerais, Bahia, São Paulo, Mato Grosso, Rio de Janeiro and Santa Catarina. In all areas visited, we set up one to eight mist nets and captured and banded hummingbirds obtaining morphometrical and other data, and collected their ectoparasites such as lice (ONIKI, 1990; 1999a, ONIKI; WILLIS, 1993), ticks (ONIKI-WILLIS; WILLIS, 2018), nematodes (ONIKI *et al*., 2002), feather mites (HERNANDES *et al*., 2016) and chiggers (ONIKI-WILIS *et al.*, 2018). Ectoparasites were collected with a forceps and placed in a glass vial with ethanol 70%, and labeled properly: individual vial for each hummingbird, with date, locality, host species' name, name of the collector, and a field number. In the laboratory, using a magnifying glass, the samples were cleaned, and separated in different arthropod orders. Cloacal temperature of one individual of *C. lucidus* was 40.4 ° C and the laboratory room temperature ranged from 25-32° C during the experiments.

For lice, eventually each sample was examined and eggs and adults were counted and many adults were mounted in slides in Canada balsam or Entellan for more detailed study.

RESULTS

From 26 July 1977 to 22 April 2001, we captured and checked 94 individuals of *Chlorostilbon lucidus* for lice in six states as follows: São Paulo State (five localities); Minas Gerais (5); Espírito Santo (4); and one locality each in the states of Mato Grosso, Rio de Janeiro and Bahia. Thus, the observations on the lice behavior and ecology come from those captures.

Case Study 1 (Sample 5024): On 23 December 1997, at Serra da Piedade, MG (19º 49' S, 43º 40' W), we captured a male Glittering-bellied Emerald. It weighed 3.8 g at 10:00 am; it had a heavy body molt. This hummingbird was infested with two species of lice, genus *Trochiloecetes* sp. and *Trochiliphagus* sp. We separated eggs, adults and feathers of the *Trochiloecetes aureoventridis* only, and called it Day 0.

The next day (24th, Day 1) in the laboratory, room temperature = 28º C, they were placed in a Petri dish to check on their survival, as follows: 28 body feathers only; 1 good egg on a feather=6; 1 good egg with one female attached=2; 1 good egg with a male=1; 1 good egg with a milky white young attached=1. Lice were alive, but they were nibbling at the feathers, seen in their digestive tract by transparency, and the good eggs that were attached to the feathers were clipped and contents sucked.

On the 25th (Day 2), at 10:00, room temperature =28.8 º C, lice were still alive but with slow movements, when touched.

On the 27th (Day 4), at 13:00, one female was dead with belly up and an egg halfway out of her abdomen; but one female and a male moved rapidly among the feathers when those were touched. They remain among the feathers as they can keep the humidity and temperature.

On the 28th (Day 5), at 15:35, Ta=32º C, one female was dead; a male was dying, only twitching the 3rd leg, while holding to the feather with mandibles.

On the 29th (Day 6), at 06:10 am, they were all dead, and placed in ethanol 70º.

Case Study 2 (Sample 5025): From a second *Chlorostilbon lucidus*, captured on the same locality and day, at 10:23; it weighed 3.5 g and we collected its lice, *Trochiloecetes aureoventridis*. On the next day (24th) (Day 1) we separated: 6 body feathers only; one feather with one female lice attached and another similar but with young ecloding; one feather with one young attached.

On the 25th (Day 2), at 10:10, room temperature =28.8 º C, lice were still alive but with slow movements, when touched, as in sample 5024.

On the 27th (Day 4), at 13:10, the ecloding young had died in that same position; one young had the belly up but holding to a feather and still alive, moving; the female was dead and it had an egg inside.

On the 28th (Day 5), at 15:45, room temperature, Ta=32º C, all lice still had slow movements when the feathers were moved, and others were dead with legs up, but some had black digestive tract, a sign that they had eaten feathers. Usually they stay under the feathers, maybe to keep temperature and humidity.

On the 29th (Day 6), at 06:10 am, they were all dead, and placed in ethanol 70º.

Case Study 3 (Sample 5405): On 20 November 2000, in Avaré, SP (23º 00' S, 48º 50' W), we captured a male *Chlorostilbon lucidus*, at 10:18. The bird weighed 3.0 g and was infested with *Trochiloecetes aureoventridis* and *Trochiliphagus* sp. We placed both species of lice in a Petri dish covered with a cloth at room temperature (Day 0). On the 24th (Day 4), at 07:30 am, one white, non-chitinized young *Trochiliphagus* sp. was still alive and moving slowly. All other adults were dead. On the 27th (Day 7), they were all dead and placed in ethanol 70º.

In general, there is one good egg and an adult or young attached to it on the ventral side of a body feather, but when there are a large population and the back of the neck (nape) becomes overcrowded, females lay two, three or even four eggs on a feather. Thus, we photographed two eggs on a feather (Figure 1) and compared their external structure to another feather with only one egg (Figures 2 and 3).

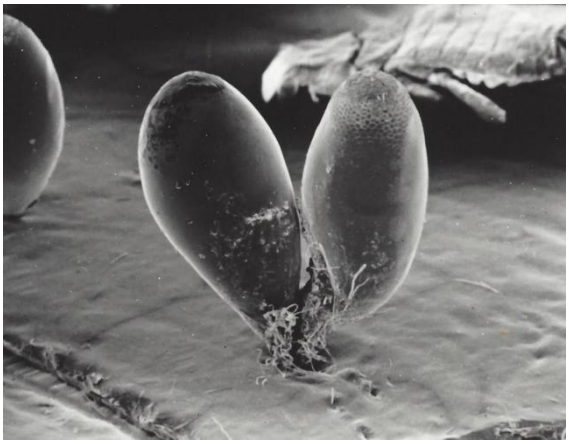

Figure 1. Two eggs of *Trochiloecetes aureoventridis* (Ricinidae), attached to a feather.

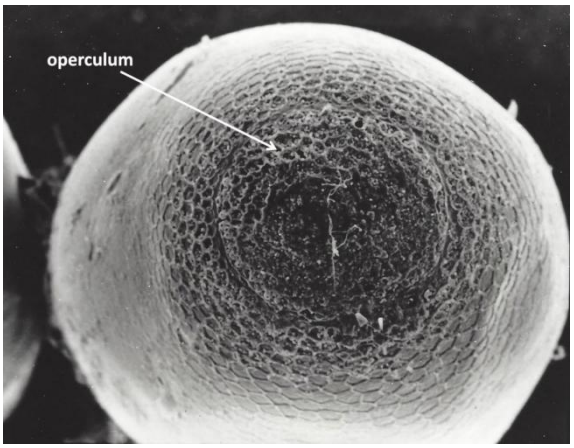

Figure 2. Detail of the top of an egg of *Trochiloecetes aureoventridis,* showing the operculum.

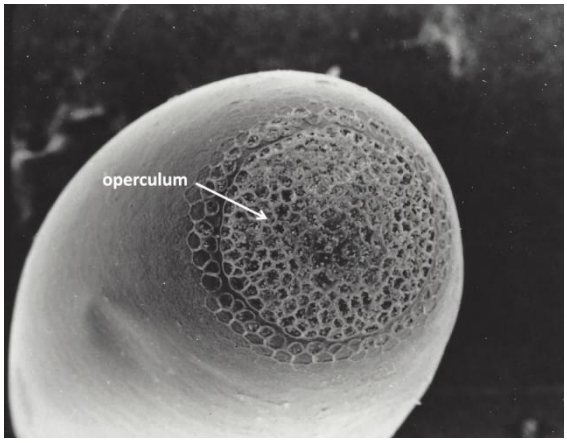

Figure 3. Top of an egg of *Trochiloecetes aureoventridis.*

The egg is a miniature, oval-shaped structure, in which the top or lid like structure called operculum, opens at the time young emerge through a structure called "hatching organ" (CICCHINO; ABRAMOVICH, 1987). Figures 2 and 3 shows a normal operculum with its mesh almost like a nice weaving only on top of the egg, and a couple more of rows of weaving outside the operculum, as it does not go down to the lateral body of the egg, *i. e*, the lateral side of the egg is smooth. The operculum is well defined and one can see a line separating this lid like structure, which opens at the time the young is emerging, which is milky white, soft and not chitinized.

Figures 2 to 4 were taken on a scanning electron microscope with eggs enlarged 200 x; except Figure 1 which was enlarged 50 x.

In addition to the three experimental studies, we had a fourth case of a *C. lucidus* that died after handling. It was captured at Avaré (SP), on 29 December 1999 (sample 5270). It had a defective bill, so it was probably weakened and his load of lice was high. It was infested with adults and young of *Trochiloecetes* sp. and *Trochiliphagus* sp.. In the laboratory, eggs, adults and young were counted (Table 1).

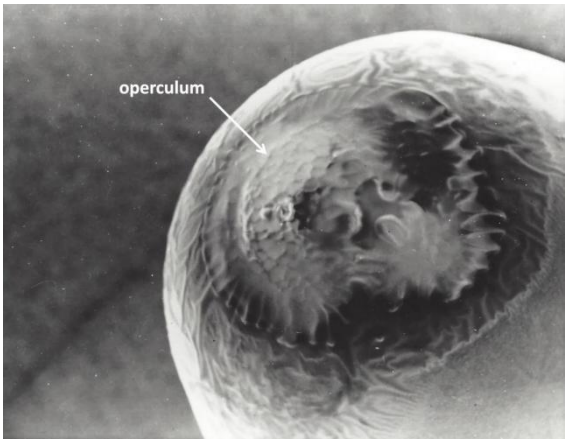

Figure 4. Detail of an egg of *Trochiloecetes aureoventridis,* with damaged operculum, due to overcrowding.

In Table 1, we call "good egg" the ones that was milky white and shiny; "empty egg" the ones that had hatched and it is empty, transparent. "Addled eggs" were found only in *Trochiliphagus* sp. but each feather had only one egg. Although they had more females, they were not laying as many eggs, but it seems they were restricted to few feathers, laying 2 eggs or more in each. Some second instar young had a reddish abdomen as it had fed on blood.

Table 1 - Data on *Trochiliphagus* (A) and *Trochiloecetes* (B) (Phthiraptera) found in sample 5270.

	Lices species	
	Trochiliphagus	***Trochiloecetes***
Feather with one good egg		1
Feather with two good eggs	3	
Feather with 1 good egg		13
Feather with empty egg		2
Feather with two empty eggs	6	
Feather with 1 empty and 1 good egg	1	
Feather with 2 empty and 1 good egg	1	
Feather with 1 addled egg	7	
Free male	3	
Free female	9	1
Young female with egg inside		1
Free young, 1st instar	13	2
Free young, 2nd instar	2	

DISCUSSION

In the four experimental cases, in the lack of the host to provide the regular blood meal, *Trochiloecetes* sp. turned to eat the feathers, parts of the eggs and even parts of another individual (cannibalism). Also, it looks like the first reaction to lack of food, the pregnant female starts laying her egg. According to Ash (1960), Phthiraptera are "very sensitive to temperature and have a narrow range of preference". This can explain, why the lice remained under the provided feathers but still did not attain the ideal temperature. Their normal habitat is within the body feathers of the birds. While the cloacal temperature of an individual *C. lucidus* was 40.4° C, and room temperature ranged from 25-32° C, it was far from the ideal temperatures for the lice. Humidity was not measured. In this way the adults and milky white non-chitinized young lasted about 6 days, at the end of which we placed them all in ethanol 70°. Even with some replacement food, they all died in 6-7 days, maybe with possibly added lack of ideal humidity and temperatures.

Although this sample 5025 was not terribly overcrowded, we could see that the "normal" eggs (Figures 2 and 3) did not develop right when there are two on a feather, as we can see in the pictures (Fig. 1, overcrowding and 4, operculum damaged). Thus, it seems that overcrowding of lice may not present a good development of the egg and fails to produce an individual. Even due to limited laying area, its is not interesting to have an extremely large population on a host.

In the case of sample 5270, as the host had a defective bill, probably it could

not preen well, so the lice was thriving and building up in their population. However, as the bird died and the blood meal host was cooling, lice was starting to reproduce as fast as they could, and eventually, many came out to the cotton holding bag. Species of these genera do not usually come out to the ornithologists' hands when handled for weighing and measuring. So, after two days, we found an unsually high number of the milky white newborn of *Trochiloecetes*, filled with red blood inside, probably from a recent feeding. Even in a young, not well chitinized female, it was possible to see the egg inside by transparency.

We found many addled eggs of *Trochiliphagus* sp. when, there is an overcrowding, and many young hatching as the ideal condition of temperature, humidity and feeding start to become critical by death of the host.

It is possible that overpopulation, exceeding the carrying capacity of their environment has a strong and devastating impact to lice due to exhaustion of resources, degradation of the environment (in this case the feathers) causing them not only malnutrition and maybe diseases.

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BIBLIOGRAPHY

ASH, J. S. A study of the Mallophaga of birds with particular references to their ecology**. Ibis**, v. 102, p. 93-110, 1960**.**

BALTER, R. S. Lice egg morphology as a guide to taxonomy. **Medical and Biological Illustration,** v. l8, p. 94-95, 1968.

BALTER, R. S. The microtopography of avian lice eggs. **Medical and Biological Illustration**, v. l8, p. l66-l79, 1968.

CICCHINO, A.C.; ABRAHAMOVICH, A. Una nueva espécie de *Trochiloecetes* Paine & Mann 1913 (Insecta, Phthiraptera, Amblycera) y características de los huevos de los espécies de este genero. **Revista de la Asociation de Ciencias Naturales del Litoral**, v. 18, n. 2, p. 175-184, 1987.

CLAY, T. Piercing mouth-parts in the biting lice (Mallophaga). **Nature**,v. 164, n. 4171, p. 617, 1949.

DALGLEISH, R. C.; PRICE, R. D. Two new species of *Myrsidea* (Phthiraptera: Amblycera: Menoponidae) from

hummingbirds (Apodiformes: Trochilidae). **Occasional Papers of the Western Foundation of Vertebrate Zoology**, v. 6, p. 1-9, 2003a.

DALGLEISH, R. C.; PRICE, R. D. A new genus and four new species of chewing lice (Phthiraptera: Amblycera: Menoponidae) from hummingbirds (Apodiformes: Trochilidae). **Occasional Papers of the Western Foundation of Vertebrate Zoology**, v. 7, p. 1-9, 2003b.

HERNANDES, F. A.; PEDROSO, L. G. A.; ONIKI-WILLIS, Y. Five new feather mites of the subfamily Pterodectinae (Acariformes: Astigmata: Proctophyllodidae) from passerines and hummingbirds (Aves) of Brazil. **Zootaxa**, v. 4161, n. 3, p. 301-328, 2016.

LYRA-NEVES, R. M.; FARIAS, A.M.I.; TELINO-JÚNIOR, W.R. Interações entre Phthiraptera (Insecta) e aves (Emberizidae) de Mata Atlantica, Pernambuco, Brasil. **Ornithologia**, v. 1, n. 1, 43-47, 2005.

LYRA-NEVES, R. M.; FARIAS, A. M. I.; TELINO-JÚNIOR, W. R.; ARZUA, M.; BOTELHO, M. C. N.; LIMA, M. C. A. E. Ectoparasitismo em aves silvestres (Passeriformes - Emberezidae) de Mata Atlântica, Igarassu, Pernambuco. **Melopsittacus,** v. 3, n. 2, p. 64-71, 2000.

ONIKI, Y. Survey of lice (Mallophaga) and some remarks on their life cycles on birds at Balbina, Amazonas, Brazil. **Revista Brasileira de Biologia**, v. 50, n. 3, p. 615-617, 1990.

ONIKI, Y. O. projeto de anilhamento de beija-flores no Brasil. **Atualidades Ornitológicas**, n. 59, p. 4, 1994.

ONIKI, Y. *Trochiliphagus austini* sp.n. (Mallophaga, Trochiloecetidae) from *Amazilia candida* (Aves, Trochilidae) of Belize, with biological notes. **Iheringia, sér. Zool.,** Porto Alegre, n. 78, p. 67-71, 1995.

ONIKI, Y. Avian parasites and notes on habits of lice from Mato Grosso, Brazil. **Iheringia, sér. Zool.** Porto Alegre, n. 86, p. 183-190, 1999a.

ONIKI, Y. Ecological differences among four genera of Phthiraptera infesting south-eastern Brazilian hummingbirds. **Wiadmości parazytologiczne**, v. 45, n. 4, p. 559, 1999b.

ONIKI, Y.; KINSELLA, J. M.; WILLIS, E. O. *Pelecitus helicinus* Railliet & Henry, 1910 (Filarioidea, Dirofilariinae) and other nematode parasites of Brazilian birds. **Mem. Inst. Oswaldo Cruz,** v. 97, n. 4, p. 597-598, 2002.

ONIKI, Y.; WILLIS, E. O. Pesos, medidas, mudas, temperaturas cloacais e ectoparasitos de aves da Reserva Ecológica do Panga, Minas Gerais, Brasil. **Bol. Centro de Estudos Ornitológicos**, SP, n. 9, p. 2-10, 1993.

ONIKI-WILLIS, Y.; WILLIS, E. O.; GOFF, M. L. New records of chiggers (Acari: Trombiculidae, Leeuwenhoekiidae) from birds collected in Brazil. **Atualidades Ornitológicas**, n. 202, p. 4-7, 2018.

ONIKI-WILLIS, Y.; WILLIS, E. O. Tick (Acarina) diversity from South American birds and mammals. **Atualidades Ornitológicas,** n. 206, p. 33-46, 2018.

PIACENTINI, V. Q.; ALEIXO, A.; AGNE, C. E.; MAURÍCIO, G. N.; PACHECO, J. F.; BRAVO, G. A.; BRITO, G. R. R.; NAKA, L. N.; OlMOS, F.; POSSO, S.; SILVEIRA, L. F.; BETINI, G. S.; CARRANO, E.; FRANZ, I.; LEES, A. C.; LIMA, L. M.; PIOLI, D.; SCHUNCK, F.; AMARAL, F. S. R.; BENCKE, G. A.;

COHN-HAFT, M.; FIGUEIREDO, L. F. A.; STRAUBE, F. C.; CESARI, E. Annotated checklist of the birds of Brazil by the Brazilian Ornithological Records Committee / Lista comentada das aves do Brasil pelo Comitê Brasileiro de Registros Ornitológicos. **Revista Brasileira de Ornitologia**, v. 23, p. 91–298, 2015.

PRICE, R. D. Order Mallophaga. Ch. 22, Pp. 215-223. *In*: Stehr, F. W.**Immature Insects**, vol.1, Dubuque, Iowa: Kendall/Hunt Publ. Co. 754p., 1987.

PRICE, R. D.; HELLENTHAL, R. A.; PALMA, R. L. World checklist of chewing lice with host associations and keys to families and genera. *In*: Price, R.D., R.A. Hellenthal, R.L. Palma, K. P. Johnson, D. H. Clayton. The chewing lice: world checklist and biological overview. **Illinois Natural History Survey Special Publication,** v. 24, p. 1–448, 2003.

RODA, S. A.; FARIAS, A. M. I. Aves silvestres infestadas por Phthiraptera (Insecta) na Zona da Mata Norte de Pernambuco, Brasil. **Revista Brasileira de Zoologia**, v. 16, n. 3, p. 871-878, 1999.

SAXENA, A. K; ARYA, G; BANSAL, N. Egg laying site and oviposition pattern of two phthirapteran species parasitizing red whiskered Bulbul (*Pycnonotus jocosus*) **Turk Journal of Parasitology**, v. 36, n. 3, p.166–168, 2012. doi: 10.5152/tpd.2012.39.

TYAGI, S.; GUPTA, N.; JOSHI, V. D.; RASHMI, A.; ARYA, G.; SAXENA, A. K. The eggshell morphology of *Heterodoxus spiniger,* infesting dog, *Canis familiaris* (Boopiidae, Mallophaga).**Journal of Applied and Natural Science***,* v. 1, n. 1, p. 71–73, 2009.

VALIM, M. P. *Craspedorrhynchus linardii*, a new species of chewing louse (Phthiraptera: Ischnocera: Philopteridae) from the Gray-headed Kite (Aves: Falconiformes: Accipitridae). **Zootaxa**, n. 1173, p. 57-62, 2006.

VALIM, M. P. Three new species of *Dennyus* Neumann, 1906 (Phthiraptera, Amblycera, Menoponidae) parasitic on swifts (Aves, Apodiformes, Apodidae) in Brazil. **Papéis Avulsos de Zoologia** (São Paulo), v. 53, n. 30, p. 415-429, 2013.

VALIM, M. P.; CICCHINO, A. C. Six new species of *Myrsidea* Waterston, 1915 (Phthiraptera: Menoponidae) from New World jays of the genus *Cyanocorax* Boie (Passeriformes: Corvidae), with notes on the chronic structure of eggs. **Systematic Parasitology**, n. 2, p. 191-211, 2015a.

VALIM, M. P.; CICCHINO, A. C. A remarkable new genus and a new species of chewing louse (Phthiraptera, Ischnocera, Philopteridae) from Brazil**. Zookeys**, n. 541, p. 57-70, 2015b.