



## A gregarine from the gut of cat flea, *Ctenocephalides felis* (Bouché) (Siphonaptera: Pulicidae) in Taiwan: Dynamic of infection patterns

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### ABSTRACT

An understanding on host–parasite interaction is essential for control of disease causing organisms in domestic animals. The cat flea, *Ctenocephalides felis* (Bouché) is the predominant flea infesting dogs and cats in Taiwan. It was collected from 933 dogs and 197 cats from Taiwan. A total of 5878 *C. felis* adults were recovered; 14.6% fleas were observed to harbor *Steinina ctenocephali*. Female fleas were more susceptible to gregarine infection than males. Further, fleas were more likely to be infected with the gregarine at high temperatures, particularly during March–July with high parasite prevalence and intensity. Fleas harboring gregarines infection were higher in dogs than cats. Our study may help in development and application of appropriate flea control measures in Taiwan.

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### 1. Introduction

The fleas belong to the Order Siphonaptera that comprises approximately 2575 species and subspecies to date (Whiting et al., 2008). They are holometabolous insects with immature stages (egg, larva and pupa), found in borrows or nests. The adult cat flea *Ctenocephalides felis* (Bouché) is a renowned pet nuisance, cosmopolitan in distribution, polyxenic and a vector of several human and animal pathogens, and has developed resistance to several families of insecticides (Krämer and Mencke, 2001; Bossard et al., 2002). In consequence, the studies of their endosymbionts have been explored as an attempt to discover potential bio-control candidates. Those studies have reported the presence of a variety of endosymbionts in *C. felis* such as *Rickettsia* like organisms, protozoans, nematodes and cestodes (Beard et al., 1990; De Avelar et al., 2007).

Gregarines exhibit monoxenous life cycle and they may be a promise as bio-control agents (Clopton and Gold, 1996). They are ubiquitous unicellular microorganisms parasitizing marine and terrestrial invertebrates. New species of gregarines and updated taxonomic arrangements are steadily increasing for example, recently a gregarine *Steinina* sp. was identified in cat fleas from Brazilian dogs and their seasonal prevalence studied (De Avelar and Linardi, 2008). The total number of gregarine species described to date is around 1600 (Adl et al., 2007). There are 6 gregarine species currently described from fleas grouped within the 6 genera (Alarcón et al., 2011). New technologies have yielded unexpected results by linking gregarines with metabolic syndrome and obesity in insects (Schilder and Marden, 2006), and placing gregarines as a sister group of the *Cryptosporidium* spp., the etiological agents of Cryptosporidiosis in humans and other animals (Leander et al., 2003; Templeton et al., 2010). In Taiwan, *C. felis* is commonly parasitizing dogs and cats, and has been a focus of several ecological studies (Shyu et al., 1993; Hsu and Wu, 2000, 2001; Hsu et al., 2002). Tsai et al. (2008, 2011) reported the presence of the human pathogen

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*Rickettsia felis* and *Bartonella henselae* in cat fleas from Taiwan. Alarcón et al. (2011) recorded *Steinina ctenocephali* (Ross) (= *Gregarina ctenocephali* Ross (1909)) for the first time from Taiwan which was originally described from Port Said (Egypt) from dogs. The patterns of infections of gregarines in cat flea have not been reported except De Avelar and Linardi (2008). We screened more than five thousands of cat fleas for gregarine infection in three years time period that provides a broader understanding on flea–gregarine relationship unlike previous studies. We focused on the cat flea–gregarine association, seasonal occurrence and fall out of infection period in Taiwan and provide an assessment on the implications of our findings.

## 2. Materials and methods

### 2.1. Flea collection, observations and terminology

Adult *C. felis* were collected by combing 933 dogs and 197 cats in an animal shelter, Taipei, Taiwan from May 2006 to May 2008 (Table 1). Dogs and cats included in the study were mostly young which are significantly more infected with cat flea in Taipei, Taiwan (Shyu et al., 1993), of short hair, of either sex. The flea's samples were taken at their arrival from naturally infected stray dogs and cats, collected by the Taipei animal control regularly throughout the year. Collection was made at weekly intervals. Type of animals (dog and cat) and date of collection were recorded. Fleas were recovered from each dog or cat by combing for 10 min effort. Live fleas were transferred to plastic tubes, labeled, and individual samples maintained from each dog and cat and taken to laboratory. Each flea was placed under microslide with saline solution for 2 min for observing gregarine infection rate. Fleas were examined using a dissecting microscope Leica Zoom 2000 (Tokyo, Japan). Infected fleas were kept alive for further experiments. The number of infected and uninfected fleas, their sexes, and developmental stage of the gregarine observed were recorded. In all the observations, only one species, *S. ctenocephali* was identified. Ecological terminologies follow Bush et al. (1997). Mean temperature, mean relative humidity and total monthly rainfall for the year 2006–2008 were obtained from the Central Weather Bureau (CWB), Taipei, Taiwan, ROC.

### 2.2. Data analysis

A generalized linear model with random intercept (glmmML) was used to evaluate factors influenced gregarine infection in fleas, the response variables being the sex of fleas, type of hosts (cats and dogs), temperature, mean relative humidity and total monthly rainfall and the gregarine infection as a random effect. A negative binomial distribution was assumed accounting for the individual host variation. The model was specifically fitted to test the role of temperature, humidity, rainfall, host type and sex of adult *C. felis* as function of the gregarine infection. Prevalence of gregarine infection in sampled fleas and the intensity of infection when possible were recorded. Positive *S. ctenocephali* infected fleas were observed in vivo and the intensity of infection estimated under stereomicroscope as described above for gregarine infection. Flea index ( $\pm$ SE), defined as the mean number of fleas per infected host (Marshall, 1981) was used. Positive *S. ctenocephali* infected fleas were observed in vivo and intensity of infection estimated under stereomicroscope as described above for whole flea samples. Correlations between the infection rate variable in dogs and cats relative to their flea index during the study were estimated by applying Pearson's equation for linear correlation (data no showed). Differences in proportions were assessed using Fisher's exact test. Statistical analyses were made within the software package R (Development Core Team, 2009).

## 3. Results

Stages of *S. ctenocephali* were found among infected fleas. Gregarines were mainly in the midgut, both firmly and singly attached to the epithelium in the trophozoite stage or free floating in gamont and gametocyst stages. In contrast, the gut was entirely filled in the gametocyst stage, when heavy infections were observed. A total of 5878 *C. felis* adults were recovered; 14.6% fleas were observed to harbor *S. ctenocephali*. The glmmML model indicated that sex of fleas, host types (cats and dogs), temperature, and rainfall but not relative humidity were important in predicting the gregarine infection in fleas (Table 2). When analyzed separately, females appeared more susceptible to gregarine infection than males (Fig. 1). Fleas harboring gregarines were infecting dogs more than cats (Fig. 2). The

**Table 1**  
Summary of the field collection data.

Hosts		Fleas				Gregarines			Proportion	
Host	THS	HF1G	Flea per sex		Total flea	FIG	FS1G		PFC	PFIG
			Female	Male			Female	Male		
Dog	933	141	3109	1147	4256	782	604 (77.2%)	178 (22.8%)	2.7:1	3.4:1
Cat	197	42	1255	367	1622	74	60 (81.1%)	14 (18.9%)	3.4:1	4.3:1
Total	1130	183	4364	1514	5878	856	664	192	<sup>a</sup> 2.8	<sup>b</sup> 3.5

HF1G, number of hosts harboring flea infected gregarine; FIG, fleas found infected with gregarines; FS1G, flea sexes infected with gregarines; PFC, proportion of fleas collected, female:male; PFIG, proportion of fleas infected with gregarines, female:male; THS, total hosts sampled.

<sup>a</sup> Obtained from total flea per sex, female: male.

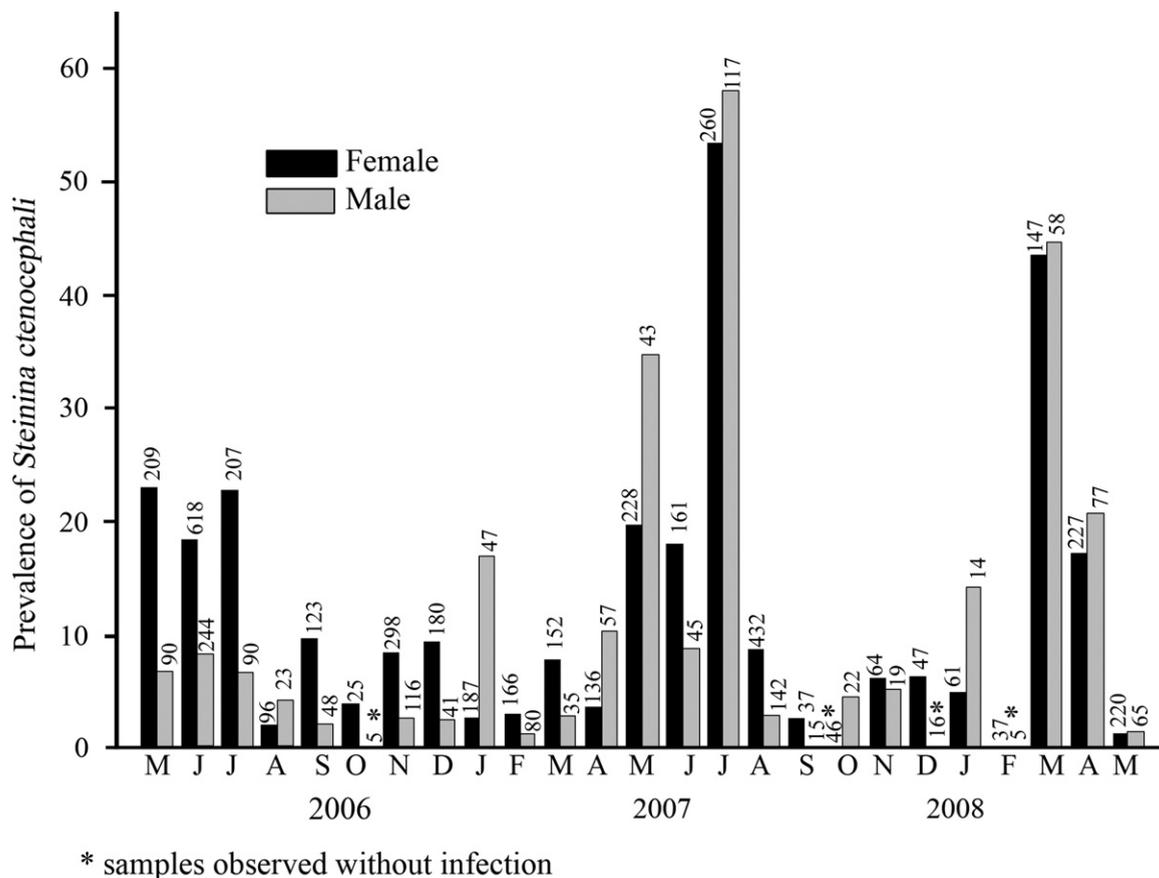
<sup>b</sup> Obtained from FS1G, female:male.

**Table 2**  
Maximum-likelihood estimates with *S. ctenocephali* infection random effect of fleas collected.

Variable level	B <sup>a</sup>	SE <sup>b</sup>	z-Value	p of z-value
Intercept	-9.41	4.69	-2.01	4.48e-02
Sex of fleas (i.e. females and males)	0.45	0.11	4.11	3.91e-05
Type of host (i.e. cats and dogs)	1.42	0.26	5.58	2.40e-08
Temperature	0.12	0.04	2.77	5.64e-03
Average humidity	0.04	0.05	0.75	4.55e-01
Total rainfall	-0.00	0.00	-2.97	3.01e-03

<sup>a</sup> Estimated coefficients.

<sup>b</sup> Standard error for the coefficient. Standard deviation in mixed distribution: 1.536; standard error: 0.1203.



**Fig. 1.** Overall prevalence of *S. ctenocephali* living in the cat flea *C. felis* from May 2006 to May 2008 as a function of the sex of infected adult fleas. Numbers over bars denote females and males observed.

correlation between the variables, prevalence of gregarines and flea index (data not showed), when studied separately for dogs and cats, was positively significant in both cases ( $r^2 = 0.5$ ,  $df = 23$ ,  $p = 0.02$  and  $r^2 = 0.64$ ,  $df = 23$ ,  $p = 0.0005$ , respectively). Thus, an increase in the number of fleas per host implied a rise in the number of infected fleas, particularly females. But we have also proved, that the fleas on cats are less frequently infected with gregarines, so the correlation results are subordinated by the proportion of infected fleas found in cats (60♀:14♂; 4.3:1) than those of dogs (604♀:178♂; 3.39:1).

Seasonality had a significant effect with fleas being more likely infected when temperatures (along with the rainfall) increases (Fig. 3). The proportion of infected fleas during that period was highly significant when compared with

other months (Table 3) (Fisher's Exact test,  $p = 7.64e-14$ ;  $7.32e-51$  and  $1.75e-05$ ). Intensity of gregarine infection was also influenced by the effect of the temperature, host type and sex of adult fleas (data not showed). It was revealed that out of 377 fleas collected in July 2007, 207 fleas were gregarine infected. The 69.6% (142/207) were heavily infected with more than 10 cells (gametocysts and trophozoite stage), and most of those fleas were females (100♀, 42♂) from dogs.

#### 4. Discussion

Cat fleas, *C. felis* in Taiwan are known to harbor the gregarine symbiont *S. ctenocephali*, whose biology and morphology was recently described (Alarcón et al., 2011) but

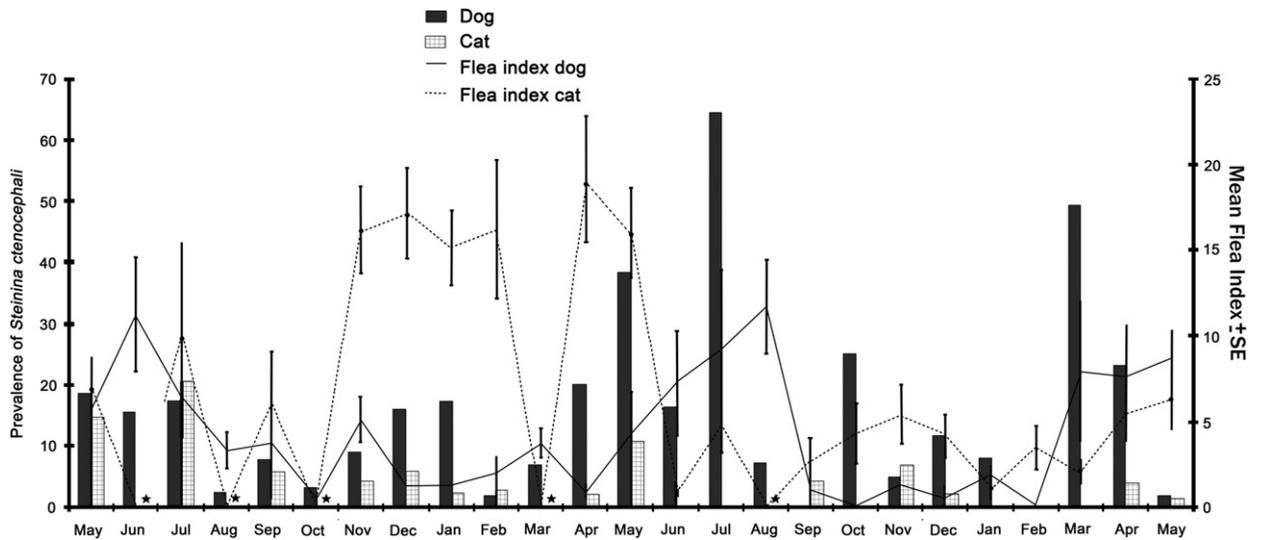


Fig. 2. Overall prevalence of *S. ctenocephali* living in the cat flea *C. felis*, from May 2006 to May 2008 as a function of host types (dogs and cats) and flea index. \* denotes data not recorded.

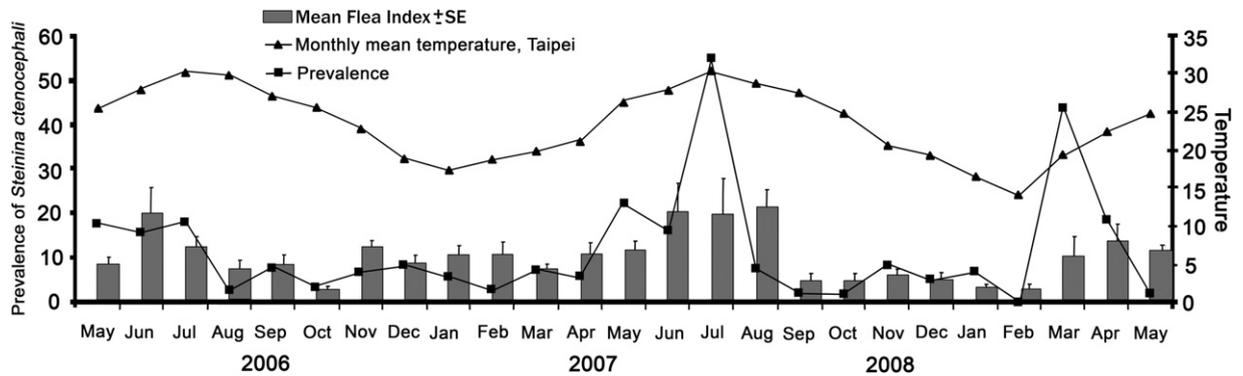


Fig. 3. Monthly prevalence of *S. ctenocephali* in cat flea *C. felis* from May 2006 to May 2008 as a function of temperature and flea index  $\pm$  SE.

much remain unknown regarding ecological aspect of this interaction. In here we have showed that temperature along with the rainfall strongly influence infection pattern of *S. ctenocephali* in *C. felis*. Temperature also increased the number of fleas on host bodies as shown by the flea index (Fig. 2) in conjunction with those living in the burrows (immature stages) (Krasnov et al., 2004a). Thus, when conditions are appropriate, an increase in the abundance of the entire flea population is expected, creating ideal conditions

for gregarine perpetuation. Shyu et al. (1993) observed that an optimal condition 20–30 °C temperature and 53–85% RH favors development of *C. felis* in Taipei. *S. ctenocephali* is transmitted from adult fleas to their progenies by means of their oocysts which are ingested by developing *C. felis* larvae. The necessity of feces of adult fleas for larval development, referred to as a kind of parental investment which is typical of *C. felis* (Hinkle et al., 1991; Silverman and Appel, 1994; Krasnov, 2008), promotes its perpetuation

Table 3

Temperature dependent comparison of *S. ctenocephali* infection of adult *C. felis* during May 2006–May 2008.

Year	Prevalence	
	Summer	Winter
2006*	May–July ( $n = 1458$ ) 16.60%	August–December ( $n = 955$ ) 6.60%
2007*	March–July ( $n = 1234$ ) 26.26%	August–February ( $n = 1320$ ) 5.38%
2008*	March–May ( $n = 794$ ) 19.02%	January–February ( $n = 117$ ) 4.27%

\*  $p < 0.05$ , Fisher's exact test.

and survival (Alarcón et al., 2011). We also found that female fleas showed a tendency to be more positive to *S. ctenocephali*. This pattern may be due to two non-mutually exclusive explanations: (i) male developing larvae present protracted development (Linardi and Nagem, 1972; Linardi et al., 1997) thus, female flea's larvae are exposed earlier to gregarine infection (oocysts) or (ii) the infection bias is driven by the proportion of female:male (3:1) in the environment increasing the chances of encounter female flea larvae–*S. ctenocephali* oocysts. Although, interpreted cautiously due to varying sample size, our data also suggest that fleas infecting (feeding) on dogs are usually hosting *S. ctenocephali*. Cats with high intensities of flea infection were coupled with months with high rates of gregarine infections, although fleas collected from cats are less likely to be infected with gregarines (Fig. 2 and Table 1). The data in here corroborates that cats are the preferred host for *C. felis* in Taipei as evidenced by the difference in the flea index (Fig. 2) on cats over dogs, which is specially clear under favorable environmental conditions for flea development (Krasnov et al., 2004b; Poulin, 2005; Krasnov, 2008). The host selection by fleas has been documented as an important factor for its fitness (egg production, viability and hatchability) and fitness cost. A significant decrease in eggs production is noticed when fleas exploiting the wrong (alternative) host (William, 1993; Krasnov et al., 2002). Under this assumption, we believe that *C. felis* exploiting dogs in Taipei as an alternative host would suffer fitness impairment. An extension of the larval stage and diminution of the feces excreted as consequence of host blood biochemistry are evoked. A suboptimal diet would hamper the opportunity for larvae to ingest available oocysts, which might explain the bias in the number of the infected fleas found on dogs as well as high frequency of infection in female fleas as above mentioned. Ross (1909) reported infection patterns of *S. ctenocephali* (= *Gregarina ctenocephali*) in cat flea, *C. felis* (= dog flea, *Ctenocephalus serripes* (Gervais, 1844)) at the rate of 38%. However, Ross sample size is unknown and his two years observations were made over the same mother and son host dogs. Our prevalence (14.6%) of this gregarine from cat flea fall within the ranges of prevalence to others gregarines found associated with this flea in USA and Brazil. Those studies have reported prevalence of gregarines at 19.4%, 11%, and 12.7% in cat fleas respectively (Beard et al., 1990; De Avelar et al., 2007; De Avelar and Linardi, 2008). De Avelar and Linardi (2008) observed significant seasonal variation of gamonts and gametocyst stages of *Steinina* sp. and suggested a synchrony between the development of *Steinina* sp. and *C. felis*. In according with our observations, under laboratory conditions, the gregarine *S. ctenocephali* does not show neither temperature-dependent change in the velocity of development or sex bias in the prevalence of infection in a ratio 1:1 (female/male). It has been already established that the survival of the *S. ctenocephali* population depends on the flea larvae encountering oocysts. Thus, the numbers of oocysts ingested is by chance, so the larger the number of oocysts available in the environment, the greater possibility of being ingested. In the field multifactorial determinants may account by the patterns observed, for example oocysts are resistant to environmental conditions

and may be dormant for long periods. Hence, to understand the spatio-temporal dynamic of this protozoan there is a need of further studies on the entire flea population along with their gregarine dynamic off and on host simultaneously.

The *S. ctenocephali* is associated with a worldwide pest, thus our finding will pay dividends in a global scale if the molecular identity of this gregarine around the world is confirmed.

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## References

- Adl, S., Leander, B., Simpson, A., Archibald, J., Anderson, O., Bass, D., Bowser, S., Brugerolle, G., Farmer, M., Karpov, S., Kolisko, M., Lane, C., Lodge, D., Mann, D., Meisterfeld, F., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S., Smirnov, A., Spiegel, F., 2007. Diversity, nomenclature, and taxonomy of protists. *Syst. Biol.* 56, 684–689.
- Alarcón, M., Huang, C., Tsai, Y., Chen, W., Dubey, A., Wu, W., 2011. Life cycle and morphology of *Steinina ctenocephali* (Ross, 1909) comb. nov. (Eugregarinorida: Actinocephalidae), a gregarine of *Ctenocephalides felis* (Siphonaptera: Pulicidae) in Taiwan. *Zool. Stud.* 50, 763–772.
- Beard, C., Butler, J., Hall, D., 1990. Prevalence and biology of endosymbionts of fleas (Siphonaptera: Pulicidae) from dogs and cats in Alachua County, Florida. *J. Med. Entomol.* 27, 1050–1061.
- Bossard, R., Dryden, M., Broce, A., 2002. Insecticide susceptibilities of cat fleas (Siphonaptera: Pulicidae) from several regions of the United States. *J. Med. Entomol.* 39, 742–746.
- Bush, A., Lafferty, K., Lotz, J., Shostak, A., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* 83, 575–583.
- Clopton, R., Gold, R., 1996. Host specificity of *Gregarina blattarum* von Siebold, 1839 (Apicomplexa: Eugregarinorida) among five species of domiciliary cockroaches. *J. Invertebr. Pathol.* 67, 219–223.
- De Avelar, D., Bussolotti, A., Ramos, M., Linardi, P., 2007. Endosymbionts of *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) obtained from dogs captured in Belo Horizonte, Minas Gerais, Brazil. *J. Invertebr. Pathol.* 94, 149–152.
- De Avelar, D., Linardi, P., 2008. Seasonality and prevalence rates of *Steinina* sp. (Eugregarinorida: Actinocephalidae) in *Ctenocephalides felis felis* (Siphonaptera: Pulicidae) from dogs captured in Belo Horizonte, Minas Gerais, Brazil. *J. Med. Entomol.* 45, 1139–1142.
- Hinkle, N., Koehler, P., Kern, W., 1991. Hematophagous strategies of the cat flea (Siphonaptera: Pulicidae). *Fla. Entomol.* 74, 377–385.
- Hsu, M., Wu, W., 2000. Effects of multiple mating on female reproductive output in the cat flea (Siphonaptera: Pulicidae). *J. Med. Entomol.* 37, 828–834.
- Hsu, M., Wu, W., 2001. Off-host observations of mating and postmating behaviors in the cat flea (Siphonaptera: Pulicidae). *J. Med. Entomol.* 38, 352–360.
- Hsu, M., Hsu, T., Wu, W., 2002. Distribution of cat fleas (Siphonaptera: Pulicidae) on the cat. *J. Med. Entomol.* 39, 685–688.

- Krämer, F., Mencke, N., 2001. *Flea Biology and Control*. First. Springer-Verlag, Berlin/Heidelberg.
- Krasnov, B., Khokhlova, I., Oguzoglu, I., Burdelova, N., 2002. Host discrimination by two desert fleas using an odour cue. *Anim. Behav.* 64, 33–40.
- Krasnov, B., Khokhlova, I., Shenbrot, G., 2004a. Sampling fleas: the reliability of host infestation data. *Med. Vet. Entomol.* 18, 232–240.
- Krasnov, B., Shenbrot, G., Khokhlova, I., Poulin, R., 2004b. Relationship between parasite abundance and the taxonomic distance among parasite's host species: an example with fleas parasitic on small mammals. *Int. J. Parasitol.* 34, 1289–1297.
- Krasnov, B., 2008. *Functional and Evolutionary Ecology of Fleas: A Model for Ecological Parasitology*. Cambridge University Press, Cambridge, 650 pp.
- Leander, B., Clopton, R., Keeling, P., 2003. Phylogeny of gregarines (Apicomplexa) as inferred from small-subunit rDNA and (beta)-tubulin. *Int. J. Syst. Evol. Microbiol.* 53, 345–354.
- Linardi, P., Nagem, R., 1972. Observações sobre o ciclo evolutivo de *Ctenocephalides felis* (Bouché, 1935) (Siphonaptera: Pulicidae) e sua sobrevivência fora do hospedeiro. *Bol. Mus. Hist. Nat. UFMG Zool.* 13, 1–23.
- Linardi, P., De Maria, M., Botelho, J., 1997. Effects of larval nutrition on the postembryonic development of *Ctenocephalides felis felis* (Siphonaptera: Pulicidae). *J. Med. Entomol.* 34, 494–497.
- Marshall, A., 1981. *The Ecology of Ectoparasitic Insects*. Academic Press, New York, 459 pp.
- Poulin, R., 2005. Relative infection levels and taxonomic distances among the host species used by a parasite: insight into parasite specialization. *Parasitology* 130, 109–115.
- R Development Core Team, 2009. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Ross, E., 1909. A gregarine parasitic in the dog-flea *Ctenocephalus serraticeps*. *Ann. Trop. Med. Parasitol.* 2, 359–363.
- Schilder, R., Marden, J., 2006. Metabolic syndrome and obesity in an insect. *Proc. Natl. Acad. Sci. U.S.A.* 103, 18805–18809.
- Silverman, J., Appel, A.G., 1994. Adult cat flea (Siphonaptera: Pulicidae) excretion of host blood proteins in relation to larval nutrition. *J. Med. Entomol.* 31, 265–271.
- Shyu, M., Hsu, T., Wu, W., 1993. Seasonal abundance of cat flea, *Ctenocephalides felis* (Bouché) (Siphonaptera: Pulicidae) in Taipei city. *Chin. J. Entomol.* 13, 59–67.
- Templeton, T., Enomoto, S., Chen, W., Huang, C., Lancto, C., Abrahamson, M., Zhu, G.A., 2010. Genome-sequence survey for *Ascogregarina taiwanensis* supports evolutionary affiliation but metabolic diversity between a gregarine and *Cryptosporidium*. *Mol. Biol. Evol.* 27, 235–248.
- Tsai, K., Lu, H., Huang, J., Wang, P., Wang, H., Huang, C., Wu, W., Shu, P., 2008. *Rickettsia felis* in cat fleas in Taiwan. *J. Vector Borne Dis.* 9, 561–563.
- Tsai, K., Huang, C., Fang, C., Shu, P., Huang, J., Wu, W., 2011. Prevalence of *Rickettsia felis* and the first identification of *Bartonella henselae* Fizz/CAL-1 in cat fleas (Siphonaptera: Pulicidae) from Taiwan. *J. Med. Entomol.* 48, 445–452.
- Whiting, M., Whiting, A., Hastriter, M., Dittmar, K., 2008. A molecular phylogeny of fleas (Insecta: Siphonaptera): origins and host associations. *Cladistics* 24, 677–707.
- William, B., 1993. Reproductive success of cat fleas, *Ctenocephalides felis*, on calves as unusual host. *Med. Vet. Entomol.* 7, 94–98.