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Siphonaptera of small rodents and marsupials in the Pedra Branca State Park, State of Rio de Janeiro, Brazil

Sifonápteros de pequenos roedores e marsupiais do Parque Estadual da Pedra Branca, Estado do Rio de Janeiro, Brasil

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Abstract

In an region of Atlantic Rainforest corresponding to the geopolitical area of the Pedra Branca State Park, Rio de Janeiro, southeastern Brazil, 160 small mammals were captured, of which 64 rodents and 96 marsupials from October 2005 to October 2007. There were collected in these hosts six flea species from three families (Ctenophthalmidae, Rhopalopsyllidae and Pulicidae), totalizing 162 specimens. *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia* was the most common species found, followed by *Polygenis* (*Polygenis*) *occidentalis occidentalis*. *Philander frenatus* and *Micoureus paraguayanus* were reported as new hosts to *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia* and *P. o. occidentalis* was reported for the first time in the city of Rio de Janeiro.

Keywords: Siphonaptera, Rodentia, Didelphimorphia, ectoparasites, fleas.

Resumo

Na Mata Atlântica área correspondente ao espaço geopolítico do Parque Estadual da Pedra Branca, Rio de Janeiro, Brasil, entre outubro de 2005 e outubro de 2007, 160 pequenos mamíferos foram capturados, sendo 64 pequenos roedores e 96 marsupiais. Nestes hospedeiros, foram coletadas seis espécies de pulgas relacionadas a três famílias (Ctenophthalmidae, Rhopalopsyllidae e Pulicidae), totalizando 162 espécimes. *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia* foi a espécie mais freqüente, seguida por *Polygenis* (*Polygenis*) *occidentalis occidentalis*. Novos hospedeiros foram registrados, *Philander frenatus* e *Micoureus paraguayanus* para *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia*, bem como a ocorrência de algumas espécies de Siphonaptera encontrados em novo espaço geopolítico, *P. o. occidentalis* e no município do Rio de Janeiro.

Palavras-chave: Siphonaptera, Rodentia, Didelphimorphia, ectoparasitos, pulgas.

Introduction

The Pedra Branca State Park (PBSP) was legally established in 1974 and is an important reference for the preservation of the remaining rainforest in the city of Rio de Janeiro. It is characterized by natural forest regrowth that occurred after intense exploration with coffee and citrus cultivation between the mid-nineteenth century and the beginning of the twentieth century. Nowadays, this park has an area of 12.5 thousand ha, reaching 1,024 m above sea level, and is one of the most important urban forest in the world, showing exuberant fragments of the Atlantic Rainforest (DEAN, 1995).

The Brazilian Atlantic forest is considered one of the ecosystems with the highest biodiversity and levels of endemism in the planet (FONSECA, 1985; BROOKS; BALMFORD, 1996; MYER et al., 2000). There is a remarkable variation in local diversity of areas along its extension. Although not many in-depth and specific studies have been conducted, the information available for some

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groups of vertebrates and invertebrates is consistent with the idea that, in Southeast Brazil, more precisely, in the latitudes of the State of Rio de Janeiro, there is a high level of diversity of species for several groups. This high biodiversity can largely be explained by the characteristics of its rough relief and edaphic particularities of the region, which enable the occurrence of habitats (ROCHA et al., 2004).

Rodents and marsupials are recognized as reservoirs of several pathogens of viral infections, helminthiasis, bacteriosis and protozoosis (AZAD et al., 1992; MILLS et al., 1995; D'ANDREA et al., 2002; HORTA et al., 2007). Given their great adaptation capacity, some of them are assiduous species in rural and urban environments and many times are associated to the transfer of pathogenic agents to domestic animals and human populations. The ectoparasites of these small mammals such as ticks, fleas and lice can play a major role as vectors of these agents (SIMON, 1954; BRASIL et al., 1989; WEBB et al., 1990; WHO, 1999; CARVALHO et al., 2001a; OLIVEIRA et al., 2008).

The order Siphonaptera consists of approximately 3,000 species of fleas of 238 genera and 15 families. In Brazil, eight families, 20 genera and 60 species have already been reported. Fleas are important invasive agents and can act as parasites per se, harming their hosts, causing irritation, and inflammatory reactions (SOUSA, 1997), but they also act as vectors, transmitting pathogens to their hosts (POLLITZER; MEYER, 1965; BARLETT; JUDGE, 1997; LINARDI; GUIMARÃES, 2000; LINARDI et al. 2005; LINARDI, 2006; MINISTÉRIO DA SAÚDE, 2008).

There is a lack of scientific information on small rodents and marsupials living in the Pedra Branca State Park, as well as on the Siphonaptera species that use them as hosts, and the study of the relationship between small mammals and their ectoparasites may broaden our understanding about their ecological, evolutionary, and taxonomic aspects. Therefore, the purpose of the present study was to evaluate the flea fauna of rodents and marsupials in the Pedra Branca State Park.

Materials and Methods

Rodents and marsupials were captured using traps of different sizes during four consecutive days and nights in each one of the 24 months of investigation, from October 2005 to October 2007, totaling 13,200 traps/day. Trapping was conducted according to Mills et al. (1995) safety recommendations. The study site is located in the Pedra Branca State Park (PBSP) (22° 56' 26" S and 43° 26' 28" W), an area of Atlantic Rainforest in the city of Rio de Janeiro, State of Rio de Janeiro. Six capture sites were delimited, beginning at into sections of altitude of 150 m up to 600 m. Traps were distributed into five transects, one of them studied each month. Banana, ground beef with bacon and peanut butter were used as bait. After capture, mammals were anesthetized with ether, ear-marked, and fleas were collected with a small tooth-comb. Fleas were preserved in 70% ethanol before being processed and mounted on glass slides for taxonomic identification. The nomenclature of Siphonaptera followed Johnson (1957) and the revision of the sub-family Rhopalopsyllinae was made according the nomenclature proposed by Linardi and Guimarães (1993). Mammal species were identified using morphological characteristics (e.g. pelage color, body and tail size) based on descriptions available for Rodentia and Marsupialia (EMMONS; FEER, 1997; MUSSER; CARLETON, 2005; WEKSLER; PERCEQUILLO, 2006).

The captured mammals were released at the same capture site. Flea species are maintained in the Siphonaptera collection of the Vector Laboratory, Sérgio Arouca School of Public Health/ FIOCRUZ.

Data on diversity and richness of small mammal species were assessed by Shanon-Wiener index (H) and Jackknife richness estimator (S) using the DIVES program (RODRIGUES, 2004) and data on parasitism were analyzed by prevalence of parasitism, Spearman's rank correlation coefficient (rs) to correlate the prevalence of fleas on hosts and altitude, coefficient of dominance, average intensity of parasitism and abundance rate. The statistical significance was set at a p-value of < 0.05 (SERRA-FREIRE, 2002; AYRES et al. 2005).

Ethical considerations: captures of small mammals were authorized by the Brazilian Institute of Environment and Natural Renewable Resources (IBAMA) (license number 058/06-RJ, process number 02022.001749/2005-64), and State Institute of Forests of Rio de Janeiro (license number IEF/RJ/PR No. 015/05).

Results

The overall diversity of small mammals estimated during the study period was H = 0.8. Considering each section studied, the highest diversity and richness were H = 0.79 and S = 13.5 at 200 m and the lowest ones were H = 0.52 and S = 6.0 at 600 and 150 m, respectively (Figure 1), totaling 160 mammals, of which 64 rodents (30 females and 34 males) and 96 marsupials (48 females and 48 males) from the following species: Cricetidae: *Akodon cursor* (Winge, 1887) (6/64), *Oligoryzomys nigripes* (Olfers, 1818) (4/64); Muridae: *Rattus rattus* (Linnaeus, 1758) (1/64), *Rattus norvegicus* (Berkenhout, 1769) (8/64); Sciuridae: *Guerlinguetus ingrami* (Thomas, 1901) (44/64); Erethizontidae: *Sphigurus villosus* (Cuvier, 1823) (1/64). The marsupials were: Didelphidae: *Didelphis aurita*

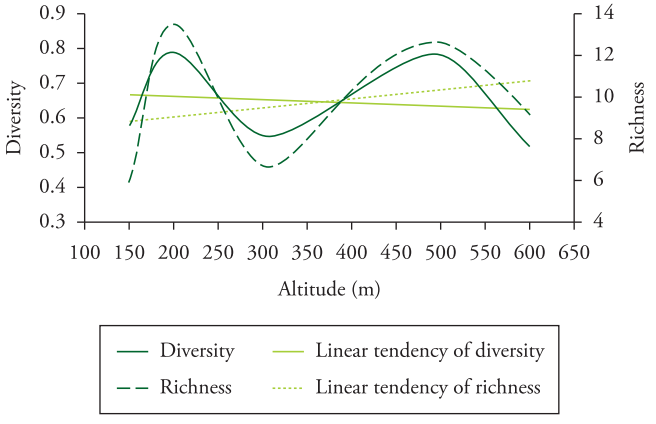

Figure 1. Shanon-Wiener diversity and Jackknife richness of small mammal species per section, Pedra Branca State Park, Rio de Janeiro, Brazil, from October 2005 to October 2007.

(Wied-Neuwied, 1826) (56/96), *Marmosops incanus* (Lund, 1840) (17/96), *Micoureus paraguayanus* (Tate, 1931) (18/96), *Metachirus nudicaudatus* (Geoffroy, 1803) (1/96), *Monodelphis americana* (Müller, 1776) (3/96) and *Philander frenatus* (Olfers, 1818) (1/96). The linear regression between richness and diversity indicated the influence of the first ecological data on the second one ($r^2 = 0.75$, $p < 0.05$) (Figure 2). There were nine recaptures during the study period: four *M. paraguayanus*, two *D. aurita*, two *M. incanus* and one *G. ingrami*, and nine specimens of *P.occidentalis* were recovered. The frequency of captured mammals and their related prevalence of parasitism in each section are shown in Table 1.

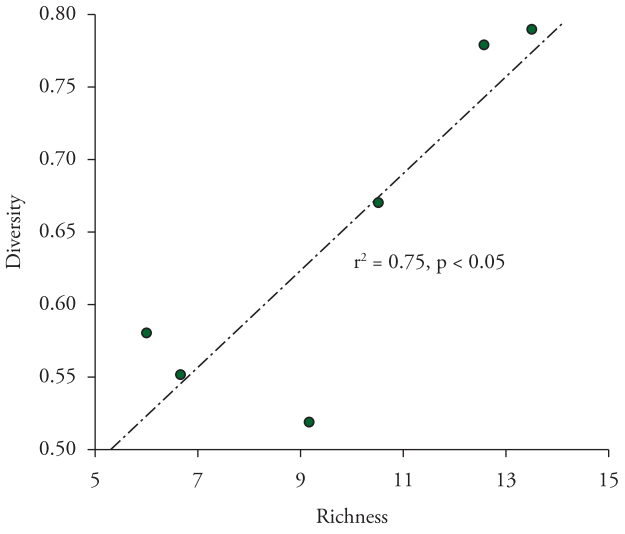

Figure 2. Goodness-of-fit linear regression between Shanon-Wiener diversity and Jackknife richness of small mammal species, Pedra Branca State Park, State of Rio de Janeiro, Brazil, from October 2005 to October 2007.

There were 162 flea specimens (78 females and 84 males, sex ratio = 0.92) exploiting small mammals in PBSP, of which 37 (22.8%) in only one rodent species, *G. ingrami* (19 females and 18 males, sex ratio = 1.05), and 125 (77.2%) in marsupials, of which *D. aurita* (50 females and 61 males, sex ratio = 0.81) was the most parasitized (68.6%). They belonged to three families and six species, and *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia* (Wagner, 1901) was the most abundant, 74.7% (121/162) of total fleas collected, and was more commonly seen on *D. aurita* (89.2%), followed by *Polygenis* (*Polygenis*) *occidentalis occidentalis* (Cunha, 1914) (19.8%; 32/162), *P.* (*P.*) *rimatus* (Jordan, 1932) (19.8%; 6/162), *Adoratopsylla* (*Adoratopsylla*) *antiquorum antiquorum* (Rothschild, 1903) (0.6 %; 1/162), *P.* (*Neopolygenis*) *atopus* (Jordan and Rothschild, 1922) (0.6%; 1/162), and *Ctenocephalides felis felis* (Bouché, 1835) (0.6%; 1/162) (Table 2).

The overall prevalence of fleas was 25.6% (41 mammals parasitized out of 160 examined). Twenty-seven out of 96 marsupials examined (28.1%), and among rodents, 14 squirrels of 64 examined (21.9%) were parasitized. Neither the prevalence found among marsupials and rodents, nor the sex ratio of fleas on their hosts nor the positive correlation seen between prevalence and altitude were statistically significant ($\chi^2 = 0.8$, $p > 0.05$; $\chi^2 = 0.8$, $p > 0.05$; $rs = 0.26$, $p > 0.05$). The average intensity of parasitism (AIP) was 3.95 fleas/host [$AIP_{rodents}$ = 2.64 fleas/rodent; $AIP_{marsupeials}$ = 4.63 fleas/marsupial], and the rate of abundance was 0.59 fleas/rodent and 1.30 fleas/marsupial.

The calculated dominance of the Ctenophthalmidae family was 75.3%, of the Rhopalopsyllidae family was 24.1%, and of the Pulicidae family was 0.6% (Table 2).

There were two types of multiple infestations, one triple on *D. aurita* and two double infestations on *G. ingrami*. The triple infestation was with *A. intermedia intermedia* and *P. o. occidentalis* and *C. felis felis*; the double infestations were with *P. occidentalis occidentalis* and *P. rimatus* and *P. o. occidentalis* and *P. atopus*.

Table 1. Species of small mammals stratified by frequency and prevalence of parasitism, captured monthly per section of the Pedra Branca State Park, Rio de Janeiro, Brazil, from October 2005 to October 2007

Mammals	Collection sites (m)																				
	150			200			300			400			500			600			Total		
	N	P	%	N	P	%	N	P	%	N	P	%	N	P	%	N	P	%	N	P	%
Rodentia	10	3	30.0	16	-	-	5	2	40	10	4	40.0	11	-	-	12	5	41.6	64	14	21.9
Akodon cursor	1	-	-	1	-	-	-	-	-	-	-	-	4	-	-	-	-	-	6	-	-
Oligoryzomys nigripes	-	-	-	2	-	-	-	-	-	1	-	-	-	-	-	1	-	-	4	-	-
Rattus norvegicus	1	-	-	3	-	-	-	-	-	1	-	-	3	-	-	-	-	-	8	-	-
Rattus rattus	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-
Guerlinguetus ingrami	8	3	37.5	10	-	-	5	2	40.0	8	4	50.0	2	-	-	11	5	45.4	44	14	31.8
Sphigurus villosus	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-
Didelphimorphia	28	7	25.0	20	8	40.0	7	1	14.3	24	7	29.2	5	1	20.0	12	3	25.0	96	27	28.1
Didelphis aurita	20	7	35.0	11	6	54.5	3	1	33.3	10	4	40.0	4	1	25.0	8	2	25.0	56	21	37.5
Marmosops incanus	4	-	-	5	-	-	3	-	-	5	2	40.0	-	-	-	-	-	-	17	2	11.7
Micoureus paraguayanus	4	-	-	2	-	-	-	-	-	9	1	11.1	1	-	-	2	-	-	18	1	5.5
Metachirus nudicaudatus	-	-	-	1	1	100	-	-	-	-	-	-	-	-	-	-	-	-	1	1	100
Monodelphis americana	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	2	1	50.0	3	1	33.3
Philander frenatus	-	-	-	1	1	100	-	-	-	-	-	-	-	-	-	-	-	-	1	1	100
Total	38	10	26.3	36	8	22.2	12	3	25.0	34	11	31.4	16	1	6.2	24	8	33.3	160	41	25.6

N = number of captured hosts; P = number of hosts exploited by fleas; % = prevalence.

Table 2. Number and frequencies of fleas collected on different small mammal species captured in the Pedra Branca State Park, Rio de Janeiro, Brazil, from October 2005 to October 2007

Flea species	Hosts														Total	
	Guerlinguetus ingrami		*Didelphis Aurita*		*Marmosops incanus*		*Micoureus paraguayanus*		*Monodelphis americana*		*Metachirus nudicaudatus*		*Philander frenatus*			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Ctenophthalmidae															122	75.3
A. (*A.*) *antiquorum antiquorum*	-	-	-	-	-	-	-	-	1	100	-	-	-	-	1	0.6
A. (*T.*) *intermedia intermedia*	-	-	108	97.3	2	100	2	100	-	-	4	100	5	100	121	74.7
Rhopalopsyllidae															39	24.1
P. (*P.*) *occidentalis occidentalis*	30	81.1	2	1.8	-	-	-	-	-	-	-	-	-	-	32	19.8
P. (*P.*) *rimatus*	6	16.2	-	-	-	-	-	-	-	-	-	-	-	-	6	3.7
P. (*N.*) *atopus*	1	2.7	-	-	-	-	-	-	-	-	-	-	-	-	1	0.6
Pulicidae															1	0.6
C. felis felis	-	-	1	0.9	-	-	-	-	-	-	-	-	-	-	1	0.6
Total	37	100	111	100	2	100	2	100	1	100	4	100	5	100	162	100
(%)	22.8		68.6		1.2		1.2		0.6		2.4		3.2			

N = number of specimens; % = frequency of fleas; (%) = frequency of hosts.

Discussion

The fauna of small mammals found in the PBSP consisted of 12 species (H = 0.8), and its diversity was influenced by richness ($r^2 = 0.75$, $p < 0.05$), although the linear tendency of these ecological parameters showed an inverse correlation, i.e., as diversity decreased richness increased (Figure 1 and 2). These findings were particularly influenced by high abundance of *D. aurita* and *G.ingrami*, species commonly found in all collection sites, which are considered opportunistic species together with the other four species, *A. cursor*, *O. nigripes*, *R. rattus*, and *R. norvegicus* (Table 1). High richness or abundance of these opportunistic or generalist species that can thrive in a wide variety of environmental conditions characterize the degree of fragmentation of the vegetation (RICKLEFES, 2003) caused by disorderly occupation of the park area as observed by Cáceres (2006). These sinantropic species, of which two are typically cosmopolitan, Roof and Norway rats, harbor various bioagents and their presence in the same wild territory promote the exchange and dissemination of infectious and parasitic agents (D'ANDREA et al., 2002; OLIVEIRA et al., 2001; MINISTÉRIO DA SAÚDE, 2002) into new hosts and environments, developing new relationships between hosts and parasites and favoring the occurrence of zoonoses and geographical epidemic spread (BARLETT; JUDGE, 1997). Nevertheless, the wild fauna found, *S. villosus*, *M. incanus*, *M. paraguayanus*, *M. nudicaudatus*, *M. americana*, and *P. frenatus*, despite their low abundance (Table 1), is important to the local environment. A governmental program of reforestation was established in this region 20 years ago (SMMA, 1999), and is now maintained and constantly supervised by the Environmental Department of the City of Rio de Janeiro. The study findings suggest it is a successful program and the local fauna of small mammals can be used as a parameter to indicate good environmental quality, as described by Zanzini (2001), and provide valuable information in landscape epidemiology studies (CORTES, 1993),

With regard to fleas, studies conducted in different Atlantic rainforest areas showed a rate of abundance that varied between 0.30 among rodents in Guaraqueçaba, in the State of Paraná (BICHO et al., 1999), and 3.06 among rodents and marsupials in Angra dos Reis, State of Rio de Janeiro (GUITTON et al., 1986). Botêlho et al. (2003) reported that Siphonaptera are parasites of rodents and marsupials in the Serra Negra Biological Reserve, State of Pernambuco, showing an average intensity of parasitism of 3.70 fleas/host, rate of abundance of 2.0 fleas/host, and the highest dominance of the family Rhopalopsyllidae, which could be explained by the fact that a higher number of rodents than marsupials were captured. The dominance of this family was also observed by Barros-Battesti and Arzua (1997), but the same was not confirmed in PBSP, where there was definitely a dominance of Ctenophthalmidae family. This difference in dominance could be due to the number of captured hosts of each order, approximately 3/5 were marsupials, but the average intensities of parasitism on rodents and marsupials were similar in both studies. In addition, given that marsupials are their preferred hosts according to Linardi and Guimarães (2000), the prevalence of parasitism in both studies was similar ($\chi^2 = 0.8$, $p > 0.05$). The overall prevalence (25.6%) found was lower than that reported in the Serra dos Órgãos National Park in the State of Rio de Janeiro where the lowest altitude studied was 800 m and the prevalence found was higher than 50%, suggesting an effect of altitude and environment quality on the prevalence of flea fauna, which increases as altitude increases (CARVALHO et al., 2001a). The present study showed no relationship between the prevalence of small mammals exploited by fleas and the altitudes studied ($rs = 0.26$, $p > 0.05$). Besides, the prevalence of sinantropic and wild mammals and the sex ratio were similar ($\chi^2 = 0.64$, $p > 0.05$; $\chi^2 = 0.80$, $p > 0.05$) (Table 1), suggesting invasion of the wild

environment by commensal mammals, but not by cosmopolitan fleas, considering that only one cat flea was found parasitizing a marsupial (Table 2). Fleas were found in all transect lines studied, which corroborates this finding.

Considering the preference of *Polygenis* for rodents as described by Machado-Alison (1962), Gomes (1969), and Linardi (1975), its occurrence on marsupials would be secondary infestations as also seen in the PBSP; however, it should be noted the fact that wild rodents were not parasitized, which could be explained by the low number of individuals captured in the traps.

According to Linardi and Guimarães (2000), *P.* (*P.*) *occidentalis occidentalis* has been reported in some states of the Northeast, Southeast and South regions in Brazil. The present study described their presence in a new geopolitical area in the state of Rio de Janeiro hitherto found only in the green coast, south of the State of Rio de Janeiro (CARVALHO et al., 2001b). There are other reports such as *Philander frenatus* and *Micoureus paraguayanus*, new hosts for *Adoratopsylla* (*Tritopsylla*) *intermedia intermedia*.

Linardi et al. (2000) reported that *C. felis felis* occurs quite frequently in *Didelphis* spp, both in Brazil and North America. However, in the PBSP, only one animal of the species *D. aurita* was infested. This flea species has as primary hosts domestic dogs and cats, where the adult species remains on the host body, leaving their hosts only to hatch. It could be inferred that, in the PBSP, marsupials, dogs and cats have not been in close contact yet.

The abundance and prevalence of *A. antiquorum* were 0.6%. This specie was captured only on *M. Americana* as described by Morais et al. (2003) in a study developed in the Atlantic forest. The abundance found is lower than that reported by Gomes (1969) also in the Atlantic rainforest and Guimarães (1972) in Northeastern Brazil.

Fleas are a reservoir of *Rickettsia typhi* and *R. felis*, which are pathogens of murine typhus and these rickettsia are associated with the most prevalent rickettsial disease in Brazil, the Brazilian spotted fever group that is endemic in the states of Espírito Santo, Minas Gerais, and São Paulo (CARDOSO et al. 2006; OLIVEIRA et al. 2008), According to Azad et al. (1992), *R. felis* is maintained in cat flea by transovarial transmission, a characteristics that is of extremely importance to identify foci of this emerging disease. Recently Horta et al. (2007) reported *P. atopus* infected by *R. felis*, suggesting that this wild flea is a potential vector and reservoir. Cat fleas and *P. atopus* have dogs, rodents, opossum and humans as potential hosts (LINARDI; GUIMARÃES, 2000; LEMOS et al., 2001; CARVALHO et al., 2001a) and are endemic in the PBSP (Table 2). The disorderly urbanization and the use of the PBSP physical area for grazing threaten the park's integrity (COSTA et al., 2001). The unplanned occupation may be facilitating the development of a new focus of infectious diseases with the involvement of these arthropod vectors. More studies are needed in this area space to further explore the risks on invasion into these areas.

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