

A ROLE OF WILD BIRD POPULATION IN THE TRANSMISSION OF PARASITIC FLATWORMS (PLATYHELMINTHES: CESTOIDEA AND TREMATODEA) IN VIETNAM'S FOREST

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Abstract. The study aimed to evaluate the role of transmitting flatworms (Cestoidea and Trematodea) of wild bird populations in three national parks of Vietnam: Xuan Son in the Northwest mountainous region, Tam Dao in the Northeast mountainous region, and Cat Ba in the Hong River Delta region. Twenty-two wild avian species of 10 families were found to contain parasitic flatworms. According to the number of avian species containing parasitic flatworms, bird families are ranking: Timaliidae recorded with 7 species > Leiothrichidae and Nectariniidae both with 3 species > Pycononotidae and Muscipidae both with 2 species > Vireonidae, Sylviidae, Picidae, Cisticolididae and Cettiidae with 1 species. According to sex, avian populations containing parasitic flatworms decrease in the order: females accounting for 43.94% of the total individuals > males for 39.40% > juveniles for 16.66%. Three bird species containing flatworms *A. pallidus*, *M. gularis* and *C. bayumas* were found in two of the studied national parks, among them, only the first species was found common to all three studied national parks. They are vectors of high significance from a parasitological point of view.

Keywords: wild birds, transmitting parasitic flatworms, national park, Xuan Son, Tam Dao, Cat Ba.

1. Introduction

Vietnam is a country with high biodiversity and a rich forest ecosystem, containing many endemic genetic resources as well as transitional genetic ones. However, like several countries with rapid economic and social growth, Vietnam is facing the risk of biodiversity degradation and ecological imbalance. Especially, in the era of globalization, the problem of transmitting and spreading diseases and parasites through

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bird and animal hosts, related to the soil environment, are a big challenge. Soil-transmitted helminthes are transmitted by eggs passing in the faeces of infected people. In conditions that lack adequate sanitation, these eggs contaminate the soil. Soil-transmitted helminthes, including flatworms (*Platyhelminthes: Cestoidea and Trematodea*), infections are caused by different species of parasitic worms (Krivolutsky *et al.* 1997, Le *et al.* 2003, Gray *et al.* 2010, WHO 2020) [1-4]. A recent analysis by Nguyen Quynh Huong *et al.* (2020) suggested not only the mixing of animal excreta in the environment but also the interspecies transmission of corona viruses, as both bat and avian coronaviruses were detected in rodent feces on wildlife farms [5].

The analysis data for this study is based on field survey data of the international program *Planetary Biodiversity Inventory: A survey of the flatworms (Cestoidea and Trematodea) from the vertebrate bowels of the Earth* (2008-2014), funded by the United States National Science Foundation. One of the objectives of the program is to carry out parasitological surveys of vertebrate hosts, including birds, in areas and types of habitats, which have never been or have insufficiently been studied from a parasitological point of view. The envisaged results of such surveys are to find and describe unknown species of cestode parasites, thus enriching the knowledge on the species diversity (Caira *et al.* 2018) [6].

The present study aimed to evaluate the role of containing and transmitting flatworms (*Cestoidea and Trematodea*) in wild bird populations in Vietnam's forests.

2. Content

2.1. Materials and methods

* *Study region*

In June and July of 2014, we studied the diversity of parasitic flatworms *Cestoidea* and *Trematodea* in bird populations in three types of habitats, which have been poorly studied from parasitological a point of view [7, 8]. The study forests were three national parks including Xuan Son National Park of Phu Tho province, from Northwest mountainous region of Vietnam, 21⁰07.87' N - 104⁰57.20' E; Tam Dao National Park of Vinh Phuc province, from Northeast mountainous region, 21° 28.41' - 105° 38.35' E; and Cat Ba National Park, the island belonging to Hai Phong city, from Hong River Delta region, 20° 47.90' N - 107° 00.29'' E [9].

* *Collecting materials*

At each of these three national parks, we stayed for 5 - 6 days, for field studies. Birds were collected from three habitat types, including scrublands, forested areas, and wetlands. Birds were collected by using ornithological mist-nets. We studied a number of individuals of each bird species occurring in the area. Therefore, the numbers of the birds, which were collected during the single collecting activity, is too small to affect local populations. It is entirely in the frames of natural mortality. This study has been authorized by the Vietnamese governing body. The bird species of interest were examined for the presence of gastrointestinal parasites and ectoparasites after euthanasia in chloroform. The contents of intestines will be studied under a stereomicroscope.

Some individuals of each bird species occurring in the area were also studied. Bird's bodies were processed for deposition in the zoological collection of the CEBRED, Hanoi National University of Education. Parasites - flatworms were isolated from the intestinal contents, fixed, and conserved in ethanol for subsequent laboratory processing and examination. Then, they were carried out in our laboratories in Vietnam, Bulgaria, and Switzerland.

*** Classification and species identification**

The classification and species identification of birds follow mainly to Vo & Nguyen (1999), Richard and Le (2018) [10, 11]. Parasites were isolated from the intestinal contents, fixed, and conserved in ethanol for subsequent laboratory processing and examination. They were carried out in the laboratories in Vietnam, Bulgaria, and Switzerland. The classification and species identification of flatworms follow mainly Nguyen (1979), Khalil *et al.* (1994), Nguyen (1995) [12, 13, 14].

*** Data analysis**

For data analysis used are the Microsoft Office Excel 2010, and the multivariate ecological research version 6. PRIMER 6 is a collection of specialist routines for analyzing species or sample abundance. This method is normally used for ecological and environmental studies [15, 16]. Species similarity index Bray-Curtis (BC_{jk}):

$$BC_{jk} = \sum \frac{|n_{ij} - n_{ik}|}{(n_{ij} + n_{ik})}$$

where BC_{jk} is species similarity between studied forests;
 n_{ik} is the number of k species recorded in i samples;
 n_{ij} is the number of j species recorded in i samples.

Hierarchical cluster analysis of bird species similarities between the studied national parks is calculated and based on Bray-Curtis indices [15, 16].

2.2. Results and discussions

2.2.1. Species composition of wild avian populations containing parasitic flatworms

A parasitological survey of vertebrates including birds and bats and invertebrates including oribatid mites (Acari: Oribatida) in three national parks have insufficiently been studied from a parasitological point of view [1, 7, 8, 17-19]. Table 1 presents a species composition of wild avian populations found to contain parasitic flatworms in their intestines. Introduced are also the distribution characteristics of birds as well as their sex and age according to the three studied national parks.

Table 1. Avian species composition containing parasitic flatworms in three national parks

Distribution's characteristics	Distribution according to national parks			Notice
	<i>Xuan Son - Northwest Mountainous region</i>	<i>Tam Dao - Northeast Mountainous region</i>	<i>Cat Ba - Hong River Delta region</i>	
Scientific names				

I. Pyconotidae				
1. <i>Allophoixus pallidus</i>	M	M, F	M, M, M, F, F,	
2. <i>Hemixos castanonotus</i>		M, M, F		
II. Vireonidae				
3. <i>Erpornis zantholeuca</i>		M, F		
III. Timallidae				
4. <i>Pomatorhinus ruficollis</i>		M		
5. <i>Pellorneum tickell</i>	M, F, (?)	F		(?) Sex not clear. Accounted as a juvenile.
6. <i>Pellorneum ruficeps</i>	F			
7. <i>Stachyris nigriceps</i>		M, F, J		
8. <i>Stachyris striolata</i>	M, F			
9. <i>Macronous gularis</i>	M, M, M, F, F, F, F		M, F, F, F, F, F, J, F, F	
10. <i>Napothera brevicaudata</i>			M	
IV. Leiothrichidae				
11. <i>Alcippe morrisonia</i>	(?)	M, M, M, J, J		(?) Sex not clear. Accounted as a juvenile.
12. <i>Ianthocincla chinensis</i> (*)		F		(*) Species were firstly discovered in Vietnam. To be investigated more.
13. <i>Alcippe rufogularis</i>	M, M, M, F, F, (?)			(?) Sex not clear.

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				Accounted as a juvenile.
V. Sylviidae				
14. <i>Neosuthora davidianus</i>		M, F		
VI. Muscicapidae				
15. <i>Cyornis bayumas</i>	F, J		F	
16. <i>Luscinia cyane</i>	F			
VII. Nectariniidae				
17. <i>Aethopyga christinae</i>	J			
18. <i>Arachnothera longirostra</i>	M, F			
19. <i>Arachnothera magna</i>	F			
VIII. Picidae				
20. <i>Sasia ochracea</i>	(?)			(?) Sex not clear. Accounted as a juvenile.
IX. Cisticolidae				
21. <i>Orthotomus atrogularis</i>	M			
X. Cettiidae				
22. <i>Abroscopus superciliaris</i>	(?)			(?) Sex not clear. Accounted as a juvenile.
Total: 10 families and 22 species	11 M-13 F- 7 J = 31 bird specimens	10 M -6 F - 3 J = 19 bird specimens	5 M -10 F - 1 J = 16 bird specimens	Total: 26 M -29 F -11 J = 66 bird specimens

Legend. Bird sex: F. Female, M. Male, J. Juvenile, (?). Sex not clear.

During the study, sixty-six avian individuals have been recorded to contain parasitic flatworms in their intestines. They belong to 22 species of 10 avian families. Analysis done showed that according to the number of species containing parasitic flatworms, the avian families are ranked in the following descending order: Timaliidae was recorded with 7 species > Leiothrichidae and Nectariniidae both with 3 species > Pycononotidae and Muscicapidae both with 2 species. The remaining five families including Vireonidae, Sylviidae, Picidae, Cisticolidae, and Cettiidae, all were recorded with 1 species (Table 1).

2.2.2. Sex composition of wild avian populations containing parasitic flatworms

Tables 2-4 presents characteristics of bird populations containing flatworms by their sex in the three studied national parks.

Table 2. The avian composition containing parasitic flatworms according to each park

Bird sex	National park			Total
	<i>Xuan Son</i>	<i>Tam Dao</i>	<i>Cat Ba</i>	
Male	11 (42.31%)	10 (38.46%)	05 (19.23 %)	26 (100%)
Female	13 (44.83%)	06 (20.69%)	10 (34.48%)	29 (100%)
Juvenile	07 (63.64%)	03 (27.27%)	01 (9.09%)	11 (100%)

Table 3. The avian composition containing parasitic flatworms according to each sex

National Park	Bird sex			Total
	<i>Male</i>	<i>Female</i>	<i>Juvenile</i>	
Xuan Son	11 (35.48%)	13 (41.97%)	07 (22.58%)	31 (100%)
Tam Dao	10 (52.63%)	06 (31.58%)	03 (15.79%)	19 (100%)
Cat Ba	05 (31.25%)	10 (62.50%)	01 (06.25%)	16 (100%)

Table 4. The avian composition containing parasitic flatworms according to each sex in three national parks and sexes in each park

Bird sex	Number and percentage of sex in three national parks	National park		
		<i>Xuan Son</i>	<i>Tam Dao</i>	<i>Cat Ba</i>
Male	26 (39.40%)	31 (46.97%)	19 (28.79%)	16 (24.24%)
Female	29 (43.94%)			
Juvenile	11 (16.66%)			
Total	66 (100%)			

In comparison with sex and populations of sixty-six avian individuals containing parasitic flatworms, they decrease in the following order: 29 females (43.94% of the

total number) > 26 males (39.40%) > 11 juveniles (16.66%). Concerning the prevalence of parasitic flatworm infections in birds, there is no clear sex difference between females and males. Particularly in Tam Dao NP, analyzed sex ratio showed that the percentage of avian individuals containing parasitic flatworms decrease in the following order: male 52.63% of the total number of birds > female 31.58% > juvenile 15.79%.

It is recorded that the rate of flatworm containing in the juvenile avian population is not high, only 16.66% of the total individuals obtained. Thus, along with biological age, the rate of bird flatworm infection increases. Our investigation was done with avian populations living in the lower shrubs close to the soil environment, and one of their food sources being soil arthropods [10, 11, 19]. Likely, these are the main ways of infection and transmission of parasitic flatworms in studied wild birds. This is also an assessment given by Gray *et al.* (2010) and WHO (2020) [3, 4].

2.2.3. Species similarity of wild avian populations containing parasitic flatworms

Analysis of the distribution of bird populations containing flatworms according to three national parks showed that they decreased in the following order: Xuan Son > Tam Dao > Cat Ba. They were found 46.97% > 28.79% > 24.24% of a total of sixty-six bird specimens, respectively (Tables 2-4). The figures show that there are clear differences in the number of birds containing flatworms between the Xuan Son National Park, in comparison with Tam Dao and Cat Ba National Parks, 31 vs. 19 and 16, respectively.

Figure 1 showed that species composition similarities between the three studied national parks are not high, ranging from 14.02% to 34.03%. The highest species composition similarity was registered between Xuan Son National Park and Cat Ba National Park, reaching 34.03%. The three common species between them are *Allophoixus pallidus*, *Macronous gularis*, *Cyornis bayumas* (13.63% of total species number).

Tam Dao National Park has a distinct difference in bird species composition compared to the two above national parks. Particularly, species composition similarity between Tam Dao National Park compared to Xuan Son and Cat Ba National Parks, reaching only 16.01%. Specifically, only just three out of twenty-two avian species identified, accounting for 13.63% of the total species number, are found common to both Xuan Son and Tam Dao National Parks, namely *Allophoixus pallidus*, *Pellorneum tickelli* and *Alcippe morrisonia*. Only the one avian species, *Allophoixus pallidus*, accounting for 4.54% of the total species number, was found common to the Tam Dao and Cat Ba National Parks. This is also the only species found in all three national parks (Xuan Son, Tam Dao, and Cat Ba National Parks).

The data obtained on the highest bird species composition similarity between Xuan Son National Park of northwest mountainous region and Cat Ba National Park of Hong River Delta region, and their three common species containing flatworm, and one bird species, namely *Allophoixus pallidus*, found in all three studied national parks, are very significant from a parasitological point of view. These are important factors that should be considered when assessing the potential for transmission and spread of parasitic flatworms and pathogens through bird populations, bats, and possibly oribatid mites as well.

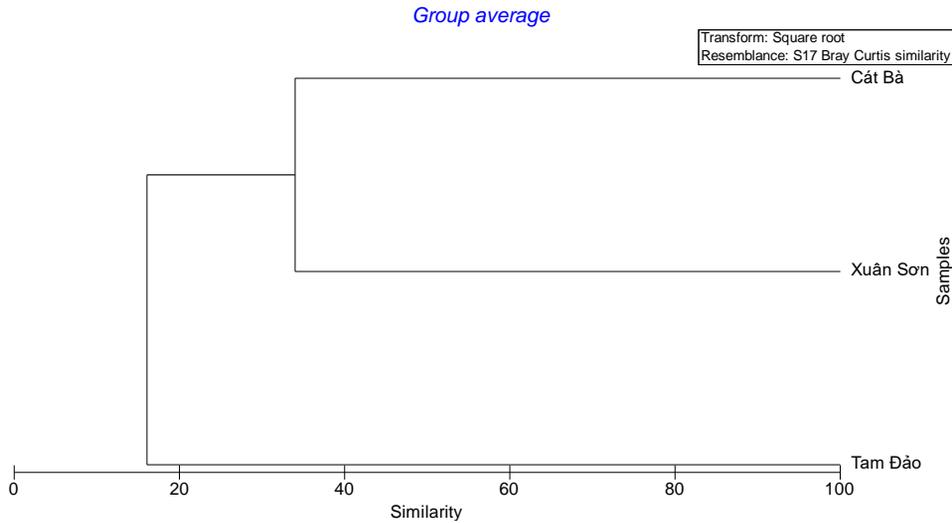


Figure 1. A cluster of species avian species similarities between three national parks (species similarities in %)

3. Conclusions

In three national parks (Xuan Son, Tam Dao, and Cat Ba National Parks), 22 wild avian species of 10 families were found to contain parasitic flatworms (Cestoidea and Trematodea). According to the species number containing parasitic flatworms, the bird families are ranking as Timaliidae recorded with 7 species > Leiothrichidae and Nectariniidae both with 3 species > Pycononotidae and Muscipidae both with 2 species > Vireonidae, Sylvidae, Picidae, Cisticolidae and Cettiidae with 1 species. By sex, avian populations containing parasitic flatworms decrease by the order: females accounting for 43.94% of the total individuals > males for 39,40% > juveniles for 16.66%.

Three bird species containing flatworms *A. pallidus*, *M. gularis* and *C. bayumas* were found in two of the studied national parks, among them, only the first species was found common to all three studied national parks. They are vectors of high significance from a parasitological point of view.

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REFERENCES

- [1] Krivolutsky D., Vu Quang Manh, Phan The Viet, 1997: The oribatid mites (Acarina: Oribatei) of Vietnam, In “*Tropical Medicine*”, Vol. I. The Russian-Vietnamese Tropical Centre, Nauka, Moscow-Hanoi, 130-145 (in Russian).
- [2] Le Thi Xuan, Malinee Thairungroj Anantaphruti, Phan Anh Tuan, Le Xuan Tu, Tran Vinh Hien, 2003. The first human infection with *Bertiella studeri* in Vietnam. *The Southeast Asian Journal of Tropical Medicine and Public Health*, 34(2), pp. 298-300.

- [3] Gray J, Zintl A, Hildebrandt A, Hunfeld KP, Weiss L., 2010. Zoonotic babesiosis: an overview of the disease and novel aspects of pathogen identity. *Ticks Tick Borne Dis.* 1: 3-10. doi: 10.1016/j.ttbdis.2009.11.003. Epub 2009 Dec 24.
- [4] WHO, 2020. Soil-transmitted helminth infections, 2 March 2020. <https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>.
- [5] Nguyen Quynh Huong, Nga NTT, Long NV, Luu BD, Latinne A, Pruvot M, Phuong NT, Quang LTV, Hung VV, Lan NT, Hoa NT, Minh PQ, Diep NT, Tung N, Ky VD, Robertson SI, Thuy HB, Long NV, Gilbert M, Wicker L, Olson SH, 2020. *Coronavirus testing indicates transmission risk increases along wildlife supply chains for human consumption in Vietnam, 2013-2014.* *Plos one*, Published: August 10, 2020, <https://doi.org/10.1371/journal.pone.0237129>.
- [6] Caira, J. N., K. Jensen, B. B. Georgiev, R. Kuchta, D. T. J. Littlewood, J. Mariaux, T. Scholz, V. V. Tkach, and A. Waeschenbach, 2017. An overview of tapeworms from vertebrate bowels of the earth. In *Planetary Biodiversity Inventory (2008-2017): Tapeworms from Vertebrate Bowels of the Earth*. J. N. Caira and K. Jensen (eds.). University of Kansas, Natural History Museum, Special Publication No. 25, Lawrence, KS, USA no. 25: 1-20. https://nhm.openrepository.com/bitstream/handle/10141/622379/2017_PBI_Cestoda_Chapt01_Overview.pdf?sequence=1.
- [7] Vu Dinh Thong, 2007. Bat conservation at Cat Ba Biosphere Reserve, North-East Vietnam, August 2006 - September 2007. Final CLP Project Report, 1-19.
- [8] Vu Quang Mạnh, Nguyen Huy Tri, Nguyen Thị Hoa, 2014. Role of oribatid mites (Acari: Oribatida) as a vector of transmission of tapeworm (Cestoda) in soil ecosystem of Cat Ba National Park, Hai Phong city, Vietnam *National University Journal of Natural Sciences and Technology*, Vol. 30, No 1S, Hanoi, 137-146 (in Vietnamese).
- [9] Bird Life International in Indochina, 2004. *Sourcebook of Existing and Proposed Protected Areas in Vietnam*. Second Edition. Hanoi, Vol. I.
- [10] Vo Quy, Nguyen Cu, 1999. *A list of Vietnam's birds*. Agricultural Publishing House, Hanoi (in Vietnamese).
- [11] Richard Crail & Le Quy Minh, 2018. *Birds of VietNam*, Lunx and BirdLife International Field Guides, Lynx Edicions, Barcelona.
- [12] Nguyen Thi Le, 1979. *The fauna of Vietnam's Cestoda - Parasites on Birds and Mammals*. DSc. Thesis. Academy of Sciences, Moscow, USSR (in Russian).
- [13] Khalil, L F, A Jones, and R A Bray (Eds), 1994. *Keys to the Cestode Parasite of Vertebrates*. Wallingford: CAB International, 1-768.
- [14] Nguyen Thi Le, 1995. *Trematodes (Trematoda) parasitizing on Vietnam's birds and animals*, H., Science and Technology Publishing House, 1-250 (in Vietnamese).
- [15] Clarke KR, Gorley R. N., 2001. Primer v5: user manual/tutorial. Primer-E Ltd. Plymouth.
- [16] PRIMER 6 for Window 2007. Version 5.2.4. <http://www.primer-e.com>
- [17] Grochovskaya Y, 1967. Insects and Mites - A vector of transmission of sickness for humans in Vietnam. DSc. Thesis. 1-433 (in Russian).
- [18] Ermilov, S.G., Anichkin, A.E. & Pal'ko, 2013. I.V. Oribatid mites (Acari) from nests of some birds in South Vietnam. *Entmol. Rev.* 93, 799-804. <https://doi.org/10.1134/S0013873813060122>.
- [19] Vo Quy, 1971. *Biology of common nesting birds in Vietnam* Scientific Techniques Publishing House. Hanoi. 1-283 (in Vietnamese).