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RESEARCH ARTICLE

THE COPEPODS (CRUSTACEAN: COPEPOD) AND FISH (OSTEICHTHYES) THAT INHABIT IN THE FLUVIAL ECOSYSTEMS FROM SANCTI SPÍRITUS PROVINCE, CUBA

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ABSTRACT

Biological control, as alternative of confrontation of vector organisms, is becoming more necessary every day due to the development of resistance to insecticides. The objetive of this investigation consisted in identifying the species of copepods and fishes that inhabit in the fluvial ecosystems of Sancti Spíritus province, with emphasis in the species with best bio regulators qualities about larval populations of culicids. The investigation comprised the period 2000-2011. In the case of copepods, they are reported new records of the genus *Mesocyclops* from Cuba, where the species identified possess good bio regulators qualities; in especial, about the first immature phase or stage of mosquitoes. In relation to fluvial fishes, there were carried out six samplings in 90 fluvial ecosystems of eight municipalities from the province, where 15 species of fishes grouped in 12 geneses and six families were identified. It was demonstrated high bio regulator capacity of the species *Gambusia punctata* and *Gambusia puncticulata*, giving evidence of changes in the populations of the provincial current fluvial ictiofauna.

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INTRODUCTION

In the last decades, mankind faces the emergence and reemergence of various infectious diseases transmitted by vectors, which have been increasing around the world, due to the growing population, the extreme environmental changes, the increase of human immigrations and air travels (Arcari *et al*., 2007; Cepero, 2012). Nowadays, these diseases have been appearing in areas where were previously controlled and eliminated (Kyle and Harris, 2008; WHO, 2009). The great epidemies of dengue and malaria occurred in the last years are an example (Cassab *et al*., 2011; Maron *et al*., 2011; Lugones and Ramírez, 2012). Moreover, the epidemies by the chikungunya virus spread by *Aedes albopictus* and *Aedes aegypti* mosquitoes in the isles La Reunion and Mauricius during 2005-2006, with an estimated of 266 000 infected persons and 248 deaths (Reiter *et al*., 2006) and the one occurred in the summer of 2007 in Italy, where more than 200 cases were diagnosed (Delatte *et al*., 2008; Dehecq *et al*., 2011). Most effective measures against vectors transmitted diseases are, undoubtedly, the control of the vector insect; for that purpose the most common action is the use of insecticides, which is controversial, because it is expensive, it induces the apparition of the resistance and contaminates the environment (Collado *et al*., 1984; Schaper *et al*., 1998; Suárez and Silva, 2002). Despite many worldwide efforts, the wader transmitters of dengue and malaria have not been eliminated.

The use of biological methods has become in an alternative option with great potentialities for mosquitoes control; in fact, the use of copepods (small crustaceans) that can be found in almost all the kinds of habitat results in unquestionable importance (Hernández and Schaper, 2000; Lardeux *et al*., 2002; Kosiyachinda *et al*., 2003). All this contrasts with the scarce knowledge about the group, mainly in tropical zones. In Mexico, several states do not have any fauna record of copepods (Gutiérrez and Suárez, 2001; Salas *et al*., 2005); where the copepods have been greatly studied in the College of

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the South Frontier (ECOSUR) Chetumal Unit and the Institute of Biology, Autonomy University of Mexico. The increase of mosquito transmitted diseases compromises every time the scientific community to prioritize the alternative searches of biological control, where the use of larviphagos fish is highlighted. This has been greatly increasing, mainly in tropical countries; where mosquito transmitted diseases constitute a scourge for human health (Ghosh *et al*., 2011). In these cases, the larviphagos fish are one of the few alternatives of control at their reach and some occasions, the only agent of biological control available (Manna *et al*., 2011; Aditya *et al*., 2012).

Together with the introduction of aloctonous species, it is now, the warming of the planet and intensification of extreme meteorological disturbance, which has brought changes in the behavior of diseases and transmitters (Gore, 2007). They are also added: the disproportionate growing of cities, the increase of number of breeding grounds, and the scarce knowledge in ecological material about culicids and methods of confrontation (Aditya *et al*., 2012).

Because the geographical location of Cuba, the climatological characteristics and archipelago with numerous small islands and open keys, is not far from the negative consequences of the introduction of exotic species in fluvial ecosystems where inhabit the native fish bio regulators of mosquito larvae, many of them, vectors of diseases, for human and animal health (Cádiz, 2005).

The investigations carried out about the fluvial ictiofauna in the centern part of Cuban archipelago are scarce and even more, the one related to the use of river fish in the control of larval populations of mosquitoes with entomoepidemiological interest.

The objective of this investigation was to identify the copepods species and fish that inhabit the fluvial ecosystems of Sancti Spíritus province, mainly the ones possessing bio regulators potentialities about larval populations of mosquitoes.

MATERIALS AND METHODS

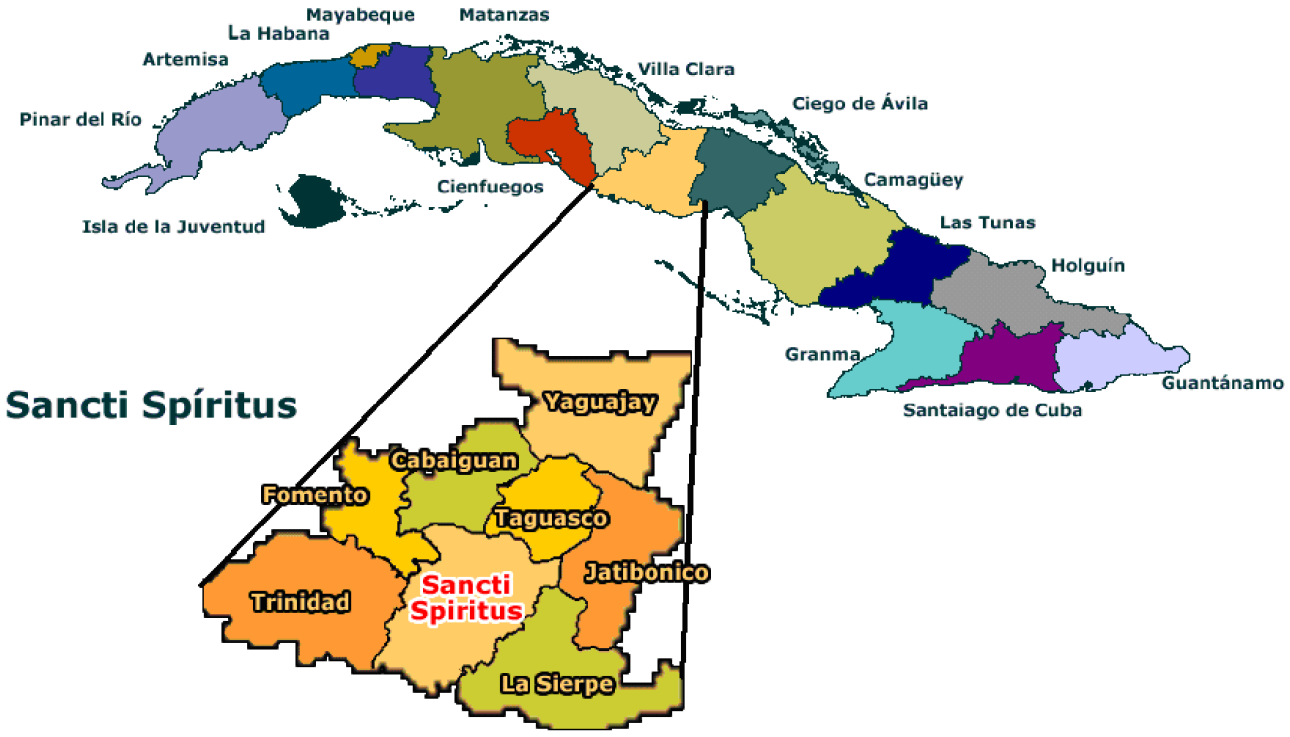
Description of the study place

Sancti Spíritus province is located in the centern part of Cuba, formed by eight municipalities: Yaguajay, Jatibonico, Taguasco, Cabaiguán, Fomento, Trinidad, Sancti Spíritus and La Sierpe. It is limited to the west of Villa Clara, to the east, with Ciego de Ávila province, to the south with Cienfuegos (Figure 1). The surface extension of the province is 6 736, 51 km^2, with a total of 462 758 habitants, for a population density of 68, 69 habitants per km^2 and has 194 population settings. The investigation comprised the period 2000 - 2011.

About copepods

Copepods specimens were collected in four municipalities of the province (Cabaiguán, La Sierpe, Sancti Spíritus and Trinidad). To collect the specimens it was used a colander of 14 x 15 x 13 cm with a net of 200 cm, the specimens were moved to the laboratory in plastic recipients with water of the own reservoirs.

In the laboratory the copepods were separated using a stereoscopic microscope of dissection Wild M5; the samples were fixed in solution of etilic alcohol to 70 % to which was added three or four drops of Koenike liquid to achieve an optima preservation of the material.



Source: Provincial Meterological Center of Sancti Spíritus

Fig. 1. Administrative Map of Cuba and Sancti Spíritus province

The samples were sent to Natural History Museum from Virginia in The United States of America, for identification. The specimens were placed in such museum. The study comprised a period of five years (2003 till 2007).

About fluvial fish

The investigation was focused in fluvial ecosystems which constitute places of ovipositor and breeding of larval populations of mosquitoes, as well as habitat of river fish. In total there were sampled 90 reservoirs. There were carried out six samples, two in the year 2000 (the first in March and the second, in June), two in the year 2005 (in March and June) and two in the year 2011 (March and August), thus there were comprised two existing season periods in Cuba (rainy: May to October and less rainy: November to April).

To collect fluvial fish in the reservoirs, it was used a colander of milimetric plastic network (1, 5 mm of diameter), with the following dimensions: 70 cm of length x 50 cm of width x 50 cm of depth, with 150 cm of handle. There were carried out three launches with the colander, ranging 2,5 m of distance between launches; always in the places of greatest presence of fish; the samples were placed in plastic recipients of 250 ml, with formol solutions of 2%, counting the quantity of collected specimens there were taken notes of the main characteristics, regarding coloration; besides, alive specimens were moved in plastic recipients and nylon bags to the laboratory of the Institute of Tropical Medicine "Pedro Kourí". To identify the collected fish there were used keys of Alayo (1973), Koldenkova & García (1990) and Jannagblin & Alvariño (1997). To determine correctly the species there were distributed the fish samples by sizes, selecting mainly the adult specimens, to have five or six specimens of the same species.

RESULTS AND DISCUSSION

There were identified four species of copepods in equal quantity of municipalities. Cabaiguán municipality resulted in being the highest number of collected specimens, as well as of the species (two), followed by Sancti Spíritus municipality. The species with the highest quantities of specimens was *M. aspericornis* (Table 1).

Table 1. Relation of copepods species collected by species and municipalities

Identified species	Cabaiguán	La Sierpe	Sancti Spíritus	Trinidad	Total
Mesocyclops aspericornis	35 females	--	10 females	--	45
Mesocyclops reidae	1 female	--	2 females and 1 male	--	4
Macrocyclops albidus	--	--	--	4 females	4
Mesocyclops pehpeiensis	--	2 females	--	--	2
Total	36	2	13	4	55

Mesocyclops aspericornis (Daday, 1906). **Sancti Spíritus**, Cabaiguán municipality. Arroyo Lajas, in temporal reservoir (pond). June 24 of 2003. Rigoberto Fimia Duarte. Tested Material: three adult females. Diagnosed by: Janet W. Reid. **Sancti Spíritus**, Sancti Spíritus municipality, pond inside the horse farm "Serafín Sánchez", in temporal reservoir (pond). August 21 of 2004. Rigoberto Fimia Duarte. Tested Material: ten adult females. Diagnosed by: Janet W. Reid.

Mesocyclops reidae Petkovski, 1986. Major synonym: *Mesocyclops ellipticus* of Smith & Fernando 1978 and Collado *et al*. 1984. **Sancti Spíritus**, Cabaiguán municipality. Arroyo Lajas, in a temporal reservoir (pond). June 24 of 2003. Rigoberto Fimia Duarte. Tested Material: a female adult. Diagnosed by: Janet W. Reid. **Sancti Spíritus,** Sancti Spíritus municipality. A pond inside the horse farm of the Institute Pre-Vocational Exact Sciences "Serafín Sánchez", Olivos I. August 21 of 2004. Rigoberto Fimia Duarte. Tested Material: two adult females and a male. Diagnosed by: Janet W. Reid.

Together with this last material collected in the pond inside the horse farm, they were also identified some Cladoceros and Ostracodas (21/08/2004). Afterwards it was carried out another sent to the Natural History Museum, next to this location (Building 31. Apartment 10, Olivos II), but the place of collection was in a plastic tank used to store up water from municipality aqueduct. Collection dates corresponded to October 6 and 18 of 2004, and were diagnosed 32 females of *M. aspericornis.*

Observations

In Sancti Spíritus province there were carried out two samples, the first one has just been detailed, in which there were identified two species, the first one (*M. aspericornis*) constituted a new record for Cuba and the second one (*M. reidae*), for Sancti Spíritus province. Next collection was made on April 16 of 2004, diagnosing in September of 2006; a female and a male, both adults and with copepodite. In that occasion, the collection was made in a lentic meander of the cited stream. Consequently, this constituted the first record of a wild species in Cuba (*M. aspericornis*), although it is supposed that must be found in several places of the Mayor and Minor Antilles. In the case of *M. reidae* was registered previously in Cuba, such as *Mesocyclops ellipticus* by Smith and Fernando (1978), who collected it in a pond from Havana province.

Macrocyclops albidus (Jurine, 1820). **Sancti Spíritus**, Trinidad municipality. Useless washing machine tank with mud and gravel at the bottom. June 2 of 2004. Rigoberto Fimia Duarte. Tested Material: four adult females. Diagnosed by: Janet W. Reid.

Mesocyclops pehpeiensis Hu, 1943. **Sancti Spíritus,** La Sierpe municipality. Pond la Maloja, almost covered of floating vegetation (*Salvinia auriculata*), temporal reservoir. August 15 of 2005. Rigoberto Fimia Duarte. Tested Material: two adult females. Diagnosed by: Janet W. Reid.

With these samples in Sancti Spíritus municipalities, it is corroborated the existing potential in Cuba since the point of view of micro crustaceans (copepods) fauna to be possibly used in mosquitoes larvae fight, either *Stegomyia aegypti* Linnaeus, 1762, or *Culex quinquefasciatus* Say, 1823 and *Anopheles albimanus* Wiedemann,1821; which confirms the stated and

tested by many investigators (Riviere y Thirel, 1981; Suárez, 1984; Kay *et al*., 1992; Marten *et al*., 1997; Reid and Suárez, 1999; de Faria *et al*., 2001).

It is necessary to have knowledge about copepods and more than that, to use adequately only the species that have bio regulator potentialities and not the ones which are intermediate hosts of a great number of parasites of the lymphatic system and of the subcutaneous tissues (group of diseases known by filariosis, caused by nematodes worms), typical example, it is the dracontiasis, where the microfilariae are developed to the point to be ineffective (one or two weeks) inside a copepod of the genus *Cyclops.*

This work evidences the need of keys to identify fluvial copepods, not only from Cuba, but also from Antilles (Smith and Fernando, 1978). Therefore, it was increased the list of river copepods species, from 26 to 27, constituting a new record for Cuba the incorporation of this species (*M. aspericornis*), which has proved to have bio regulator qualities against mosquitoes larvae of first stage, proven and demonstrated fact in various parts of the world (Vu *et al*., 2000, Kay and Vu, 2005; Marten and Reid, 2007), who refer deaths between 70 - 86 % using 25 copepods.

In relation to the results about fluvial fish, are shown below. In six samplings carried out in 90 fluvial ecosystems were possible to identify 15 species of fish, gathered in 12 genus and six families (Table 2).

Table 2. Fluvial Ictiofauna of Sancti Spíritus, according to species, condition and family

Species	Condition	Family
Gambusia punctata (Poey, 1854)	E	Poeciliidae
Gambusia puncticulata (Poey, 1854)	N	Poeciliidae
Girardinus denticulatus (Garman, 1895)	E	Poeciliidae
Girardinus falcatus (Eigenmann, 1903)	E	Poeciliidae
Girardinus metallicus (Poey, 1854)	E	Poeciliidae
Limia vittata (Guichenot, 1853)	E	Poeciliidae
Poecilia reticulata (Peters, 1895)	N	Poeciliidae
Xiphophorus maculatus (Geinther, 1866)	I	Poeciliidae
Cyprinodon variegatus (Poey, 1860)	N	Cyprinodontidae
Cubanichthys cubensis (Eigenmann, 1903)	E	Cyprinodontidae
Cichlasoma tetracanthus (Cuvier y Valenciennes, 1831)	N	Cichlidae
Tilapia rendalli (Boulenger, 1897)	I	Cichlidae
Clarias gariepinus (Burdrell, 1882)	I	Ictaluridae
Dormitator maculatus (Bloch, 1792)	N	Eleotridae
Betta splendens (Regan, 1884)	I	Osphronemidae

Legend. E: Endemic; I: Introduced; N: Naturalized.
Source: Laboratory of Biological Control of the Vice direction of Parasitology of the Institute of Tropical Medicine "Pedro Kourí" (IPK).

From the six identified families, the Poeciliidae was the best represented and distributed when being present in 100% of the municipalities studied (Table 3). From the total of identified species, the greatest quantity corresponded to the endemic and naturalized (11), which coincides with the ictiofauna of South America and the Caribbean Isles, and corroborates the theory of Iturralde and MacPhee (1999) in relation to the origin of Cuban flora and fauna, which have been demonstrated by Rodríguez (2001) for South America and Rojas *et al*. (2004) in Perú.

The municipalities where were collected a greater number of fish specimens resulted in Sancti Spíritus, Yaguajay, Cabaiguán and Fomento; that is, municipalities with coastal ecosystems, pre mountainous and mountainous, where undoubtedly, the variety of ecosystems is greater, which gives more possibilities for organisms biodiversity (Achá and Fontúrbel, 2003; González, 2006; Cassab *et al*., 2011). In the case of the municipalities that took the first two places (Sancti Spíritus and Yaguajay), the number of fluvial ecosystems sampled (29 y 20) was superior to the rest of the municipalities. There were collected a total of 15 260 of fish specimens, from which 10 068 were females (66, 0 %) and 4 710 (30, 8 %) males. It is necessary to take into account that the exotic species *T. rendalli* and *C. gariepinus* were not determined the sex (482 specimens), because the total of collected specimens were alevines and youthful; that is, they were in immature phases of their biological cycle, which makes difficult the sex determination (Bernardo *et al*., 2003; Hernández *et al*., 2006). The number of identified individuals in the 90 ecosystems sampled in different years showed a total of 6 348 specimens in the year 2000, equivalent to 41, 5% of the collected total (15 260). In 2005 the number increased to 7 561, for 49, 5%, meanwhile in 2011, this number decreased rapidly, to only 1 351 specimens, for 8, 8%.

In the case of species by municipalities, resulted in firstly *P. reticulata* (31, 7%), followed by *G. punctata* (27, 4%), then *Girardinus metallicus* (12, 1%), fourthly, *Limia vittata* (10, 7%) and then *G. puncticulata* (7, 2%), the species best represented and distributed. The greatest values of numbers of individuals by species, regarding municipalities, corresponded firstly to Sancti Spíritus (4 923/32, 2%), secondly Yaguajay (2 824/18, 5%), Cabaiguán (2 007/13, 1%) and Fomento (1 643/10, 7%). They are confirmed Yaguajay municipalities (12 species), Fomento and Sancti Spíritus (both with 10 species) as well as the greatest riches of species, all this can be appreciated in Table 4.

Table 3. Distribution of fluvial fish families by municipalities

Municipalities	Identified Families						Total
	Poeciliidae	Cyprinodontidae	Cichlidae	Ictaluridae	Eleotridae	Osphronemidae	
Yaguajay	X	X	X	-	-	-	3
Jatibonico	X	-	-	-	-	X	2
Taguasco	X	-	-	-	-	-	1
Cabaiguán	X	-	-	-	-	-	1
Fomento	X	-	X	X	-	-	3
Trinidad	X	X	-	-	X	-	3
S. Spíritus	X	-	X	X	-	X	4
La Sierpe	X	-	X	X	-	-	3
Total	8	2	4	3	1	2	20

Source: Laboratory of Biological Control of the Vice direction of Parasitology of the Institute of Tropical Medicine "Pedro Kourí" (IPK).

Table 4. Distribution of collected fish specimens by species and municipalities in six samples carried out. 2000, 2005 and 2011

Fish Species	Municipalities								
	1	2	3	4	5	6	7	8	Total
Gambusia punctata	995	205	424	144	721	240	1156	303	4188
Gambusia puncticulata	280	37	0	7	195	86	471	37	1113
Girardinus denticulatus	63	0	0	0	0	0	0	0	63
Girardinus falcatus	117	0	0	1	18	0	0	0	136
Girardinus metallicus	466	0	249	58	197	0	859	22	1851
Limia vittata	527	0	161	18	193	63	551	134	1647
Poecilia reticulata	4	373	628	1769	187	229	1471	189	4850
Xiphophorus maculatus	152	0	0	0	26	0	93	0	271
Cyprinodon variegatus	0	0	0	0	0	380	0	0	380
Cubanichthys cubensis	91	0	0	0	0	0	0	0	91
Cichlasoma tetracanthus	37	0	0	5	28	0	66	19	155
Tilapia rendalli	60	0	5	5	57	0	136	3	266
Clarias gariepinus	32	0	0	0	21	0	118	45	216
Dormitator maculatus	0	0	0	0	0	30	0	0	30
Betta splendens	0	1	0	0	0	0	2	0	3
Total	2824	616	1467	2007	1643	1028	4923	752	15260

Legend: 1: Yaguajay, 2: Jatibonico, 3: Taguasco, 4: Cabaiguán, 5: Fomento, 6: Trinidad, 7: Sancti Spíritus, 8: La Sierpe

G. punctata was the species best divided and distributed in Sancti Spíritus reservoirs before the year 2000, followed by naturalized species *P. reticulata* and *G. puncticulata* (Morejón, 1992). In this study, it was *P. reticulata* and *G. punctata* passed to second position, but most notorious and alarming was that *G. puncticulata* took fifth place, after *G. metallicus* and *L. vittata* and this, was one of the species more abundant and best distributed in our country some years ago (García and González, 1986; Hernández *et al*., 2006), this fact was corroborated by the drastic decrease of specimens of such species by samples, of 1 040 in four samples of the years 2000 and 2005 went only to 73 in two samples of the year 2011, such decrease is attributed to, regarding the number of specimens and presence in the ecosystems of *G. puncticulata,* to the substancial increase in the levels of contamination of Sancti Spiritus fluvial ecosystems (home activity, agriculture and the industries) and exacerbation of the interspecific competence, mainly, with the exotic species introduced in these fluvial ecosystems, mainly, *T. rendalli* and *C. gariepinus*, which possess a major ecological plasticity and capacity of adaptation than the native species (Ishikawa *et al*., 2010; Adytia *et al*., 2012 and Argota *et al*., 2012). In the case of exotic species introduced in the breeding grounds where ovipositioned and breed mosquitoes (*B. splendens*, *C. gariepinus, T. rendalli* and *X. maculatus*), the third of these was present in six municipalities, meanwhile the second one was collected in four municipalities; *X. maculatus*, in three and only in Jatibonico and Sancti Spíritus municipalities, the species *B. splendens*. Yaguajay, Fomento and Sancti Spíritus municipalities showed a greater number of species, as well as the values of abundance or relative densities regarding to the introduced species (with three of the four collected ones).

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