

FAUNA AND DISTRIBUTION OF SANDFLIES (DIPTERA, PHLEBOTOMIDAE) IN YUGOSLAVIA, CROATIA, MACEDONIA AND THEIR ROLE IN THE TRANSMISSION OF PARASITIC AND VIRAL DISEASES

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(Received, 23. February 1998.)

*The first investigations of phlebotomine sandflies in Yugoslavia were started in 1900, but more detailed faunistic research did not take over until 1928. In this paper special attention has been paid to very wide ecological investigations in the period 1969-1990. It is worth noting that in this period important virological investigations were carried out, especially in the species *Phlebotomus perilliewi*, Parrot 1930. Also important were investigations on these insects in the endemic foci of visceral leishmaniasis in Serbia, as well as in Montenegro, Croatia and Macedonia.*

Key words: Phlebotomine sandflies fauna, ecology, viruses

INTRODUCTION

Sandflies belong to the medically important species of haematophagous insects, attracting great attention in former Yugoslavia. They are vectors of the causative agents of sandfly fever and also of cutaneous and visceral leishmaniasis. These diptera roused special interest in the period immediately following World War II, because at the time the mentioned diseases (with the exception of cutaneous leishmaniasis) acquired alarming proportions in some parts of the country.

Detailed faunistic investigations of sandflies all over former Yugoslavia were begun in 1947 and subsequently, when new cases of visceral leishmaniasis were detected in children in 1968, an extensive ecological study was started in 1969, which included the following: the density of the mixed populations of sandflies, faunistic composition, relative abundance of various species, diurnal and seasonal fluctuations of their populations, imago behavior, the sex ratio and the age of female specimens, and determination of the origin of blood meals as well as isolation of some viral strains. It is for these reasons that these investigations are of great importance in the epidemiology of diseases in which sandflies act as vectors.

Sandflies were studied in endemic foci of visceral leishmaniasis in East and South-East Serbia, in Montenegro around Bar and in some coastal places from

Ulcinj to Prevlaka (Boka Kotorska). In Croatia these diptera were studied in the coastal zone from Prevlaka to Trogir and on the island of Mljet. The last was the first investigation of its kind in this area. Over 80% of the island populations were seropositive for the viruses of sandfly fever. In Macedonia sandflies were examined in the districts of Skopje, Kumanovo and Veles.

MATERIAL AND METHODS

The sandflies were collected by hand in settlements and by sticky traps in various types of microhabitats.

For viral investigation live sandflies were captured by capturator in human settlements, from places such as bedrooms and school latrines and school latrines and in artificial light. They were transported in ice to the laboratory where they were classified and grouped into pools. Afterwards they kept frozen at -66°C.

Viruses were identified by examination of infected mouse brains by electron microscopy, by indirect immunofluorescent technique, and by complement-fixation and hemagglutination-inhibition tests (Gligić et al., 1982, 1985).

The serum proteins of the hosts to some species of sandflies were detected by the method of immunoprecipitation in 1% agar gel (Mišćević, 1980).

RESULTS AND DISCUSSION

Sandflies were studied all over former Yugoslavia but mostly in South-East Serbia, especially in the area of Dobrić, since this area was still a focus of visceral leishmaniasis.

Detailed faunistic investigations of sandflies in South-East Serbia were begun in 1947 and subsequently, when new cases of visceral leishmaniasis were detected in children in 1968, an extensive ecological study was started in 1969.

In former Yugoslavia the fauna of Phlebotomidae comprises 2 genera (*Phlebotomus* and *Sergentomyia*) and 10 species: *Phlebotomus papatasi*, *Phlebotomus sergenti*, *Phlebotomus neglectus* (major), *Phlebotomus perfiliewi*, *Phlebotomus perniciosus*, *Phlebotomus tobbi*, *Phlebotomus simići*, *Phlebotomus chinensis balcanicus*, *Sergentomyia minuta* and *Sergentomyia dentata*. In the examined areas of Serbia, seven species of *Phlebotomus* and one of *Sergentomyia* were found as follows: *Phlebotomus papatasi*, *Phlebotomus perfiliewi*, *Phlebotomus neglectus* (major), *Phlebotomus tobbi*, *Phlebotomus simići*, *Phlebotomus sergenti*, *Phlebotomus chinensis balcanicus* and *Sergentomyia minuta*.

The investigations of sandflies during the period from 1969-1990 were carried out in settlements and outside them (Figure 1). They covered both human dwellings and animal quarters and in artificial light at night.

Outside settlements, sandflies were caught in two types of microhabitats, such as rodent burrows (*Apodemus sylvaticus*) and cracks of supporting stone

walls along the roads and also in various biotopes: forests, cornfields, vineyards, melon fields and in manure. The results obtained from the mentioned types of microhabitats indicate that they were not only diurnal resting sites but also possible breeding sites of sandflies, because the great number of males and the relatively high percentage of fed and gravid females suggest that the insects develop fully in the holes and cracks of the supporting walls, i. e. that these are their breeding places (Živković and Mišćević, 1975; Mišćević, 1982).

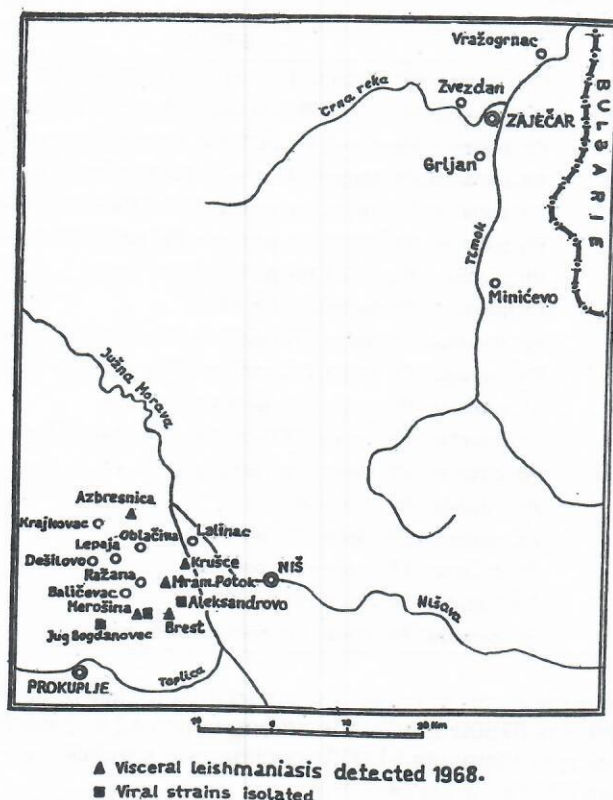


Figure 1. Area (East and South-East Serbia) where sandflies were investigated in the period 1969-1990.

Phlebotomus papatasi and *Phlebotomus perfiliewi* were regularly found in the investigated areas and the remaining species in order of frequency were: *Phlebotomus neglectus* (major), *Phlebotomus simići* and *Phlebotomus tobbi*, while *Phlebotomus sergenti* and *Phlebotomus balcanicus* were found in negligible number (Mišćević and Milutinović, 1983, 1987).

Out of the 18 investigated settlements of South-East and East Serbia the faunistic composition of sandflies was the richest in Jug Bogdanovac and Azbresnica, villages in South-East Serbia where all species were detected, as well as in Minićevo in East Serbia. A negligible number of species was found in Baličevac, Aleksandrovo, Lalinske Pojate, Krušce and Zvezdan (Table 1) (Živković and Mišćević, 1972, 1973; Mišćević, 1983).

Table 1. Detection of various species of sandflies in the period from 1968-1990 in the 18 investigated villages situated in the areas of Dobrić and Zaječar.

VILLAGES	SPECIES
Azbresnica	Ph. papatasi, Ph. sergenti, Ph. major, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Krajkovac	Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Dešilovo	Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Jug Bogdanovac	Ph. papatasi, Ph. sergenti, Ph. major, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Oblačina	Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Lepaja	Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Rožina	Ph. papatasi, Ph. major, Ph. perfiliewi i Ph. simići.
Baličevac	Ph. papatasi, Ph. perfiliewi i Ph. tobbi.
Merošina	Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Brest	Ph. papatasi, Ph. major, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Aleksandrovo	Ph. papatasi, Ph. major i Ph. perfiliewi.
Mramorski Potok	Ph. papatasi, Ph. sergenti, Ph. perfiliewi, Ph. tobbi i Ph. simići.
Krušce	Ph. papatasi, Ph. major i Ph. perfiliewi.
Lalinske Pojate	Ph. papatasi, Ph. perfiliewi.
Grljane	Ph. papatasi, Ph. major i Ph. perfiliewi.
Vražognac	Ph. papatasi, Ph. major i Ph. perfiliewi.
Zvezdane	Ph. papatasi, Ph. major i Ph. perfiliewi.
Minićevo	Ph. papatasi, Ph. major, Ph. perfiliewi i Ph. balcanius.

The total number of sandflies collected in settlements during the period from 1969-1999 was 67590 specimens among which 42 956 were *Phlebotomus perfiliewi*. Outside settlements 51 000 specimens of sandflies were collected, of which 98% were *Phlebotomus perfiliewi*.

During the same period the mixed sandfly population in settlements was the most abundant in 1974 and 1975, and then rapidly decreased in 1976. It was slightly increased in number in 1978 but during the period from 1979-1990 the number of these diptera considerably decreased.

Phlebotomus perfiliewi was predominant in the settlements in 1974. Namely, the greatest population density was attained by the species of *Ph. perfiliewi* and *Ph. Papatasi* followed by *Ph. neglectus (major)*, *Ph. simići*, *Ph. tobbi* and *Ph. sergenti*.

Outside settlements the dynamics of the population of these diptera, namely *Ph. perfiliewi*, was uniformly spread between 1972-1985. A considerable

fall in the population abundance occurred from 1978-1980, 1982-1984. and 1988-1990.

A study of the origin of blood meals in these insects is important for determination of the attraction of the various species of sandflies (as vectors of diseases) towards man and some kinds of animals. These may be hosts and reservoirs of causative agents which is of special interest to medicine. On the basis of our investigations, it might be concluded that the abdominal contents of females depend on the locality where they are collected, as well as on the hosts accessible to them, which is particularly true of *Ph. perfiliewi*.

Within the examined period of 21 years, 3 150 female sandflies which contained fresh blood were collected. Out of this number 2100 specimens belonged to the species *Ph. perfiliewi*, 800 to *Ph. papatasi*, and 350 to *Ph. neglectus (major)*. In 99% cases *Ph. papatasi* had fed on human blood, while *Ph. perfiliewi* contained blood from more than three hosts. The species *Ph. neglectus (major)* had consumed blood of man and rat. As for the latter, it is important that they are transmitters of the causative agent of kala-azar to man (Mišćević, 1977, 1980).

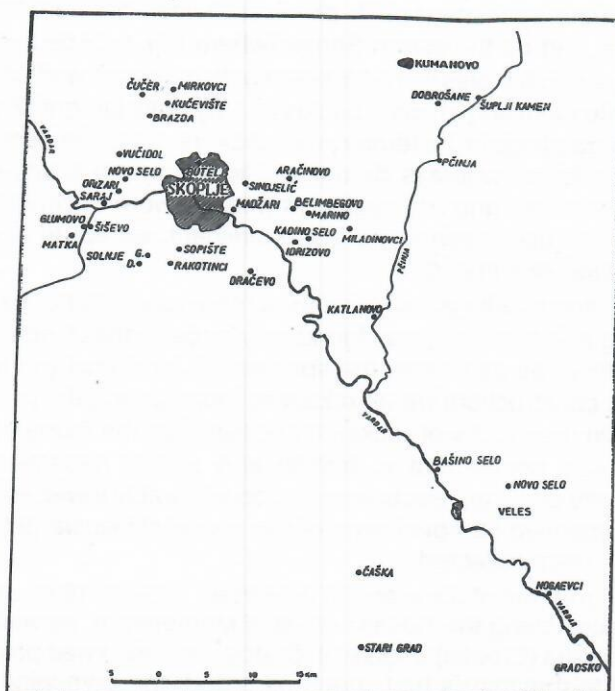


Figure 2. Part of North Macedonia with examined places.

Between 1970-1981 sandflies were found in 31 localities out of 37 investigated places around the towns of Skopje, Kumanovo and Veles (Figure 2). In the area of Veles the abundance of these insects was very low. Such a state is explained in the first place by the higher standard of living of the inhabitants of this region.

In the investigated parts of Macedonia the following sandflies were found: *Ph. papatasi*, *Ph. perfiliewi*, *Ph. neglectus (major)*, *Ph. tobbi*, *Sergentomyia minuta*, *S. dentata bruchoni* and *Phlebotomus* of the group *chinensis*.

The most frequent and abundant forms were those of the group *chinensis* as well as the species *Ph. perfiliewi*, whereas the presence and abundance of other species was much lower.

S. minuta and *S. dentata bruchoni* were found only in the area of Skopje. *S. dentata bruchoni* (which in former Yugoslavia occurred only in Macedonia) was rarely found, mainly under the eaves of roofs, in porches and exceptionally in sleeping quarters. The species *S. minuta* was more numerous and very often found also in human dwellings. In most places it comprised over 30% of the total of all other species.

Besides *Ph. simiçi* and *Ph. chinensis balcanicus* other forms of the group *chinensis* were found as well. The species *Ph. simiçi* was the most numerous; whereas only several specimens of *Ph. chinensis balcanicus* were found. In some parts of western and north-eastern Serbia (where this subspecies is the most abundant) males and females were collected both in human and animal habitats as well as outside them and in some places also by artificial light (Živković, 1967, 1969). In human dwellings in the territory round Skopje and Kumanovo all species of sandflies were found whereas by artificial light only the forms of the group *chinensis*, *Ph. perfiliewi* and *Ph. neglectus (major)* were observed. In all cases sandflies of the group *chinensis* were predominant, except in some localities where *Ph. perfiliewi* dominated.

In uninhabited buildings sandflies were more rarely found. Except that the species *Ph. neglectus (major)*, was found only once, in these objects the forms of the group *chinensis* as well as the species *Ph. perfiliewi* (on tombs and in buildings under construction) were exclusively found or only the group *chinensis* was collected (in the cracks of rocks). It appears that the fauna of sandflies in these objects was poorer than in human and animal habitations. Here the population density of these insects was also considerably lower. However, in the cemetery in Kumanovo 52 specimens of the group *chinensis* (94.2%) and *Ph. perfiliewi* (5.8%) were collected.

In the second half of June and in July from 1969 to 1985, sandflies were collected in places along the Adriatic coast of Montenegro, as well as southern and central Dalmatia (Croatia) (Figure 3). Special attention was paid to the areas where visceral leishmaniasis had been recorded: the community of Bar (Montenegro) where 10 cases of kala-azar had been recorded in 1967, and in the districts of Makarska, Omiš and Split (Dalmatia) where 6 cases of the same disease were recorded in 1968. In addition to the study of sandflies, investigations

of kala-azar in dogs were carried out in 3 localities in Montenegro (Bordoški and Savin, 1970)

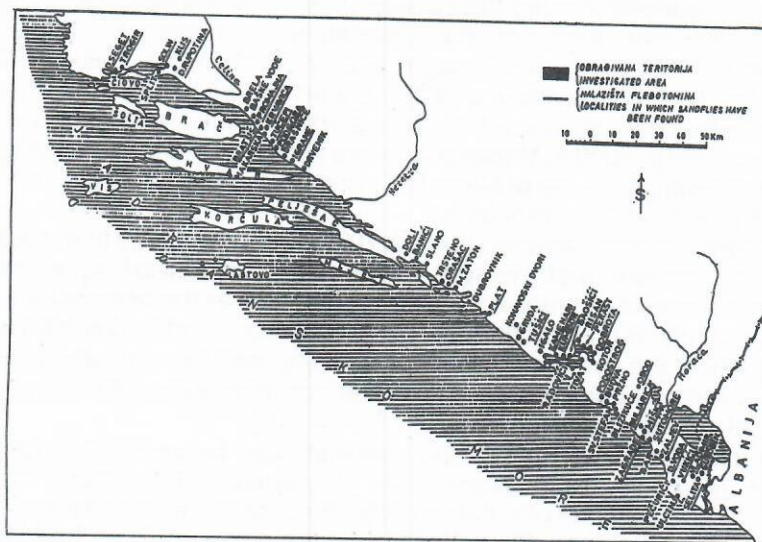


Figure 3. Investigated area of Montenegro and Dalmatia.

Out of 62 investigated locations sandflies were found only in 36 small places. In other places no sandflies were found owing to regular spraying with insecticides especially in tourist resorts. In the investigated area the following species were found: *Ph. papatasi*, *Ph. neglectus (major)*, *Ph. perfiliewi*, *Ph. tobbi* and *S. minuta*.

In dwellings *Ph. papatasi*, the species predominant in the majority of localities, was always present. Next to it, *Ph. neglectus (major)* had the highest population density; it was also frequent but was usually less numerous than the former species. However, a very high percentage of this species, in comparison with *Ph. papatasi* and *Ph. tobbi*, was collected in bedrooms in two places in Dalmatia (83% to 84,2%). The species *Ph. tobbi* was found in a smaller number of places and its abundance was considerably lower. Out of 36 localities in which the presence of sandflies was established, *S. minuta* occurred in 4, always in dwellings. Finally, a few specimens of *Ph. perfiliewi* were collected in bedrooms in a single place (Donji Seget).

In various ruins, deserted houses, buildings under construction, cracks in rocks etc. the same species as in the dwellings, except for *Ph. perfiliewi* and *S. minuta*, were found. In the above mentioned places, *Ph. neglectus (major)* was

the predominant species (especially in Montenegro); *Ph. tobbi* was considerably less frequent, while only a few specimens of *Ph. papatasi* were found (in buildings under construction only).

The results of these investigations performed between 1969-1985 indicate that out of 10 species forming the sandfly fauna, only 4 species (*Ph. papatasi*, *Ph. neglectus (major)*, *Ph. tobbi* and *S. minuta*) in Montenegro and 5 (the above mentioned 4 species from Montenegro and *Ph. perfliewi*) in Dalmatia-Croatia were found. The representatives of the *chinensis* group (*Ph. simići* and *Ph. chinensis balcanicus*) as well as the species *Ph. sergenti* and the subspecies *S. dentata bruchoni* present in Macedonia, were not found along the Adriatic coast. Therefore the sandfly fauna of Macedonia and South-East Serbia is richer than that of the investigated coastal area.

On the basis of the results obtained in this study of sandflies from 1969-1980 the authors conclude that the species *Ph. neglectus (major)* is the vector of *Leishmania donovani*. Moreover it may be considered as the only vector in some areas, particularly in the southern part of Montenegro and in Central Dalmatia (Croatia), where the species *Ph. neglectus (major)* was the most abundant from 1970-1980 and sporadical cases of kala-azar occurred and on the islands about 21 cases of cutaneous leishmaniasis.

On the island of Mljet sandflies were studied between 1985-1990. The *phlebotomus* species were examined in 10 places: Polače, Blato, Kozarica, Govedari, Soline, Babina Kuća, Babino Polje, Prožura, Pomena and Korita (Figure 4). Three places (Pomena, Blato, Kozarica) were observed to be free from any sandflies. The insects were sought inside human dwellings, under artificial light in the village and in their microhabitats close to homes, namely, supporting stone walls called "barbakan".



Figure 4. Island of Mljet showing places where sandflies were investigated.

The *phlebotomus* fauna of Mljet was composed of the following species: *Ph. papatasi*, *Ph. sergenti*, *Ph. neglectus (major)*, *Ph. perfliewi*, *Ph. tobbi*, *Ph. perniciosus* and *S. minuta*. The richest fauna was encountered at Govedari and Prožura, and then in Babino Polje, Babina Kuća and Soline. Under artificial light the greatest number of specimens was collected at Babino Polje. In human

dwelling the most abundant were *Ph. papatasi* (73.44%), *S. minuta* (13.83%) and *Ph. neglectus (major)* (6.70%). Under artificial light the most abundant was *S. minuta* (71.52%). followed by *Ph. neglectus (major)*, *Ph. perilliewi* and *Ph. papatasi*. Microhabitats (supporting walls) were populated with *S. minuta* (Biševac et al., 1990).

It should be noted that the species *Ph. papatasi* is considered a "domestic" species in the continental parts of the country. It usually populates human dwellings and, on a smaller scale, domestic animal quarters (Mišćević and Milutinović, 1986). On the island of Mljet, however, this species is attracted by artificial light, as is the case in Crimea (Petrišćeva, 1949, 1955; Žogolev, 1968). In the regions of South-east and East Serbia, a dominant place is assumed by the species *Ph. perilliewi*, both in and out of the settlements, followed by *Ph. papatasi* and *Ph. neglectus (major)*, the latter being more abundant in East Serbia (Mišćević and Milutinović, 1983; Milutinović et al., 1989). On the other hand, Mljet is dominated by two species, *Ph. papatasi* and *S. minuta*, the latter being greatly abundant under artificial light and in the microhabitats.

More detailed information of investigations into these species in Dalmatia can be found in the papers of Tartaglia (1936, 1957, 1962) and Simić and co-workers (1950, 1951). The first mentioned authors studied the insects in some coastal places and in the majority of the islands during the periods 1934-1939 and 1945-1953. However, Tartaglia was not so much concerned with the ecology but with the epidemiology of kala-azar. Simić and co-workers studied sandflies in a number of places, mostly in the coastal zone of Dalmatia (middle and north Dalmatia). Živković and Mišćević (1970) acquired data on the sandflies of south and middle Dalmatia, where five species were encountered. Their results coincided with those of Simić and co-workers and Tartaglia alike.

Since 1976, detailed viral investigations have been undertaken in relation to these diptera in South-East Serbia (Dobrić).

From the species *Ph. perilliewi* three viral strains of high contagiousness to laboratory animals have been isolated. One of these three strains was identified as the Naples sandfly fever virus (*Banyaviridae, Phlebovirus*) (Gligić et al., 1982). The identification of the second is under way and the third virus was identified on the basis of the morphology observed by electron microscopy, as well as serological findings. It has been confirmed as a new member of the *Vesicular Stomatitis* virus serogroup of *Rhabdoviridae: Vesiculovirus*, named Jug Bogdanovac virus (Gligić et al., 1981, 1983; Tesh et al., 1983). It is worth mentioning that these investigations were the first of their kind in Yugoslavia.

A c k n o w l e d g e m e n t: This work was supported by a grant from the Scientific Research Foundation of Serbia.

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FAUNA I DISTRIBUCIJA FLEBOTOMINA (DIPTERA, PHLEBOTOMIDAE) U JUGOSLAVIJI, HRVATSKOJ I MAKEDONIJI I NJIHOVA ULOGA U PRENOŠENJU PARAZITSKIH I VIRUSNIH OBOLJENJA

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SADRŽAJ

U radu je dat prikaz faune i distribucije flebotomina u jugoistočnoj Srbiji, Makedoniji, obalskom delu Crne Gore, Dalmaciji i ostrva Mljet i uloga ovih diptera u prenošenju parazitskih i virusnih oboljenja. Takođe je od značaja istraživanje ovih insekata u endemskim žarištima visceralne lajšmanijaze u Srbiji.