

P. B. Šivickis Laboratory of Parasitology of the Nature Research Centre, Vilnius: Main recent contributions to the basic parasitology studies

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In 2012, Lithuanian scientists and society at large celebrated the 60th anniversary of the Laboratory of Parasitology and the 130th birth anniversary of its founder academician of the Lithuanian Academy of Sciences professor P. B. Šivickis, who launched traditions of broad basic parasitology research in Lithuania. This paper describes main achievements of the current staff of the P. B. Šivickis Laboratory of Parasitology at Nature Research Centre (Vilnius) in basic parasitology studies, particularly molecular and morphological characterization of eukaryotic parasites.

Key words: P. B. Šivickis Laboratory of Parasitology, basic parasitology, parasite molecular and morphological characterization, PCR

In spite of huge efforts of biomedical sciences, parasitic diseases remain a severe health problem worldwide. The majority of well-established parasitic infections such as arbovirus infections, tuberculosis, bacillary dysentery, pneumococcal pneumonia, salmonellosis, hydatid disease, neurocysticercosis, malaria, leishmaniasis, schistosomiasis and many others still exist and require massive funding for their control and treatment, particularly due to the development of resistance by many strains of parasites to commonly used therapeutic drugs. Furthermore, changes in environmental conditions, including the climate change, contribute to spreading new parasitic diseases.

The World Health Organization has identified over 30 new etiological agents, and the diseases they cause, that have all appeared on the world health during last 30 years (Esch, 2004). Among them are the following eukaryotic parasites: *Cryptosporidium parvum*, *Enterocytozoon bieneusi*, *Cyclospora cayentanensis*, *Encephalitozoon hellem*, *Encephalitozoon cuniculi*, *Babesia microti*. To this group *Pneumocystis carinii*, *Capillaria philippensis*, and *Toxoplasma gondii* might be included, even

though the last species is certainly not new. They are frequently referred to as 'opportunistic pathogens'. In parallel, some 'old' parasitic infections are still flourishing. For example, about half a billion people in countries with warm climates are exposed to endemic malaria, which was estimated to cause about 2 million deaths per year, of them about 1 million are children (Snow et al., 1999).

Imported human malaria cases are known all over the world, including Lithuania. It is less known that active transmission of avian malaria takes place in Europe, including major parks of Vilnius and other European cities. Consequences of malarial infections for wildlife are insufficiently understood. For instance, as an outcome of the introduction of avian malaria parasite *Plasmodium relictum* and its competent vector in Hawaii in the 19th century, the mortality of resident birds increased up to 90% and many native species became extinct (Valkiūnas, 2005). Globally, the genus *Plasmodium* might contain several thousand species of parasites infecting birds of most species. Many malaria parasite species are cosmopolitan; that provides excellent opportunities to use these pathogens as model organisms for the study of environmental change in association with invasive species.

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It is worth mentioning that over 40 million people have been estimated infected with trematodes causing liver and lung infections (Esch, 2004). The number of reports of such traditional helminthiasis as fascioliasis and anisakiasis has increased significantly in human beings since 1980. Even developed countries are not always able to prevent the great losses and mortality caused by well-established agents of parasitic diseases. An epidemic of drug-resistant tuberculosis (Stokstad, 2000) and an outbreak of encephalitis caused by West Nile (Enserink, 1999) virus in and around the New York City are the examples. Research institutions and health agencies certainly need to be improved permanently all over the world to control parasitic infections. Well-educated staff of researchers is essential for that.

Single parasitology studies, mainly on helminthology, have been carried out in Lithuania since the 18th century (Biziulevičius, 1999). However, the broad scale fundamental and applied research on many groups of eukaryotic parasitic organisms has been first initiated in the middle of the 20th century due to initiatives and efforts made by academician of the Lithuanian Academy of Sciences, professor Pranciškus Baltrus Šivickis (1882–1968). One hundred-thirtieth birthday jubilee of this prominent biologist was celebrated in Lithuania in 2012 (Arnastauskienė et al., 2012). Overall, P. B. Šivickis supervised over 20 doctoral dissertations in parasitology; all these studies launched new branches of parasitology research in Lithuania. P. B. Šivickis' experience culminated in the monograph –“Identification of Parasites“ – (1956), which was awarded the Lithuanian State Award in Science.

The contribution of P. B. Šivickis is invaluable for establishment of the broad basic and applied parasitology research in Lithuania. Information about his life and scientific activities is available in numerous publications (see Arnastauskienė et al., 2012) and can be found in this issue of the journal. It is less known that the first Laboratory of Parasitology, which was established by academician P. B. Šivickis in Lithuania 60 years ago, is named in his honour, continues fundamental parasitology research on eukaryotic parasitic organisms at the Nature Research Centre (Vilnius), maintains traditions of basic parasitology and is known in the international context. The aim of this article is to specify main achievements of this laboratory staff in basic parasitology research during last 10 years, particularly in the field of application of the innovative polymerase chain reaction (PCR)-based tools.

Parasite DNA analyses using PCR-based methods currently are in progress in all prominent laboratories all over the world. Researches in the P. B. Šivickis Laboratory of Parasitology are visible in investigation of some groups of parasitic eukaryotic organisms in the international content due to combining modern molecular technologies and molecular characterization of parasitic organisms, on the one hand, and the deep basic traditional parasitology knowledge, particularly on parasite taxonomy, morphology, life cycles and virulence, on the other hand. It is worth mentioning that with the development of science and technologies the importance of science branches, which are capable to estimate the environment state, is growing; it is hardly feasible to reach such environment estimations without precise identification of living organisms, including identification of parasite species. Systematics, as a tool of cognition of classification, diversity and evolutionary processes in living organisms, is the basis for the development of conservation strategy and assessment of global processes, so it is of a great social and political value. Regrettably, the number of systematics specialists is declining all over the world; molecular and genetic studies are more and more often done using incorrectly identified research objects. As a result, the significance of scientific studies is often depreciated or the findings of costly investigations lose their reliability; that currently is a frequent case in parasitology (Valkiūnas et al., 2008a). That is why taxonomy research is in progress in the P. B. Šivickis Laboratory of Parasitology; several such prominent studies were carried out during last 10 years and are visible in the international content.

Two monographs, which overview results of the original research and literature data on world faunas of protists and helminths, were published and have no analogues in the current literature. First, Bondarenko and Kontrimavičius (2006) summarised results of their long-time original and parasite collections studies on fauna and biology of avian helminths of the family Aploparaksidae (Cestoda).

This book contains description and illustrations of over 90 helminths species and provides original information on distribution and biology of the parasites, including original information on life

cycles of over 40 species. Second, Valkiūnas (2005) has generalized the fundamental data on malaria and related haemosporidian parasites (order Haemosporida) of birds on the basis of the original investigation and analysis of collection materials and the literature; there are data on over 200 species of bird haemosporidians. In both books, the basic information is given on the history, classification, life cycles, specificity, pathogenicity, distribution, ecology and some other aspects of the parasites' biology. For all taxa, the most important taxonomic literature, author, date of description, synonyms, diagnosis, invalid parasite names are given. Also given are the vertebrate hosts, invertebrate hosts, type material, place of deposition of the type material, etymology, description, and illustrations for most of the species. Both books contain information on methods of the parasites' research and identification. The keys for identification of families, genera and species of these parasites have been compiled, providing opportunity for taxonomic research on these parasitic organisms.

Younger generations of parasitologists currently are obligatory trained in fundamentals of parasite biology, particularly taxonomy, in parallel with PCR-based methods in the P. B. Šivickis Laboratory of Parasitology; that provides opportunities for maintaining basic knowledge on parasite biology in the laboratory as well as to plan and carry out research aiming at targeting application of molecular markers in parasite taxonomic, genomic, ecology and evolutionary biology studies. The following main PCR-based studies have been published by researchers in the P. B. Šivickis Laboratory of Parasitology during last 10 years.

Over 30 new species of eukaryotic parasites (protists and helminths) have been described; the majority of them belong to the genera *Plasmodium* (malarial parasites), *Haemoproteus*, *Trypanosoma*, *Aploparaksis* and *Arostrilepis*. Importantly, the molecular markers (mainly sequences of mitochondrial cytochrome *b* gene) were developed for the majority of these parasite species identification for the first time (Križanauskienė et al., 2006, 2010, 2012; Valkiūnas et al., 2009, 2011; Iezhova et al., 2010, 2011; Makarikov et al., 2011; Ilgūnas et al., 2013). Additionally, molecular characterization of over 20 species of haemosporidian parasites has been developed for the first time (Hellgren et al., 2007; Levin et al., 2011; Sehgal et al., 2011;

Ilgūnas et al., 2013). **It provides new opportunities to apply molecular markers in various fields of parasitology research, particularly the parasites' epidemiology and ecology studies.**

The difficulty in identification of different ontogenetic forms of parasite species calls for search of new characters, which are independent of morphology. Molecular methods based on DNA sequencing were used to characterize adult and larval stages of flatworms belonging to the Trematoda and to clarify life-cycles of some of these parasites (Petkevičiūtė et al., 2004, 2010, 2012). The results of comparative molecular analysis uncovered genetically distinct lineages within a well-known species of Trematoda, *Bunodera lucioperca*, suggesting the existence of cryptic speciation (Petkevičiūtė et al., 2010). The use of molecular markers opened new perspectives in establishing species-specific characters for confident identification of sphaeriid bivalves and in elucidating the phylogenetic relationships among their species (Petkevičiūtė et al., 2006; Stunžėnas et al., 2011).

Analysis of cestodes of shrews (genus *Sorex*) shows that the fauna of these parasites likely is older than the contemporary fauna of their hosts in the Palaearctic; that warrants further investigation (Binkienė et al., 2011).

An international zoogeography and phylogeography study on avian blood parasites has been launched due to initiative of the laboratory (see Valkiūnas et al., 2003). The prevalence and genetic diversity of haemosporidian parasites (order Haemosporida, genera *Plasmodium* and *Haemoproteus*) infecting house sparrows (*Passer domesticus*) were examined on the global scale. This bird is native to the Mediterranean region and other areas with warm summer climates in Europe and Central Asia from where it expanded its range into Northern Europe during the Bronze Age. Over the last two centuries and with human assistance the house sparrow spread to all continents (except Antarctica) and many oceanic islands. Over 1 800 individuals of house sparrows were sampled at 58 locations on 6 continents; the birds were tested for haemosporidian parasites both by microscopic examination of blood films and PCR-based methods (Marzal et al., 2011). The results of this study show that haemosporidian parasites in the house sparrows' native range (the Mediterranean region) are replaced by species from local host-generalist parasite fauna in

the alien environments of North and South America. Furthermore, in colonized regions sparrows displayed a lower diversity and prevalence of parasite infections. Because the house sparrow lost its native parasites when colonizing the American continents, the release from these natural enemies may have facilitated its global invasion during the last two centuries.

Experimental haemosporidian vector research on the level of parasites' genetic lineages has been initiated and is in progress. Recent experimental studies show that patterns of sporogonic development of the closely related lineages pSGS1 and pGRW11 and different strains of the lineage pSGS1 of *P. relictum* are similar, indicating that phylogenetic trees based on the *cyt b* gene likely can be used for predicting sporogonic development of genetically similar avian malaria lineages in mosquito vectors (Kazlauskienė et al., 2013).

Experimental studies on virulence of malaria parasites have been also carried out (Palinauskas et al., 2008, 2011). It was shown that the virulence of the same genetic lineages of malaria parasites is markedly different in different avian hosts. Additionally, simultaneous *P. relictum* and *Plasmodium ashfordi* infections are highly virulent and act synergetically during primary infections in some but not all passerine birds. That should be taken into consideration in bird conservation studies.

Modern genetic methods have been combined with innovative laser micro-dissection techniques in the laboratory studies (Palinauskas et al., 2010).

That provides an opportunity to start genomic research of intracellular blood parasites. For the first time the staff of the laboratory developed a method that generates large amount of purified avian haemosporidian DNA (Palinauskas et al., 2013). The method is based on a unique biological feature of haemosporidian parasites, namely that mature gametocytes can be readily induced to exagellate *in vitro*. That results in the development of numerous microgametes, which can be separated from host blood cells by simple centrifugation. Our study revealed that this straightforward method provides opportunities to collect pure parasite DNA material, which can be used as a template for various genetic analyses including whole genome sequencing of haemosporidians infecting birds and lizards (Palinauskas et al., 2013). Genomic studies of avian haemosporidians, which aim to reach better un-

derstanding speciation processes in these parasites (Valkiūnas et al., 2008b), are in progress in the laboratory.

Academician P. B. Šivickis launched a tradition in Lithuania to participate in field-work abroad, including tropical countries. This tradition is maintained by the current laboratory staff. Numerous publications have been recently published using original material collected during expeditions of the laboratory staff to Europe, Asia, Africa, South America (Bondarenko, Kontrimavičius, 2006; Chasar et al., 2009; Križanauskienė et al., 2010; Loiseau et al., 2010; Levin et al., 2011; Sehgal et al., 2011; Smith et al., 2011, and many others).

In conclusion, 3 monographs, 1 book chapter and over 70 papers on basic parasitology were published by the staff of the P. B. Šivickis Laboratory of Parasitology due to collaboration with prominent international publishers and journals during last 10 years, testifying to the enthusiastic work of the researchers who maintain traditions, which were launched by academician P. B. Šivickis in Lithuania over 60 years ago.

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GAMTOS TYRIMŲ CENTRO P. B. ŠIVICKIO PARAZITOLOGIJOS LABORATORIJA: PAGRINDINIAI DABARTINIAI PASIEKIMAI FUNDAMENTINĖS PARAZITOLOGIJOS SRITYJE

Santrauka

2012 metais Lietuvos mokslininkai bei mokslo visuomenė paminėjo Gamtos tyrimų centro Parazitologijos laboratorijos įkūrimo jubiliejų ir 130-ąsias Lietuvos mokslų akademijos akademiko, profesoriaus P. B. Šivickio gimimo metines. Akademikas P. B. Šivickis padėjo pagrindus fundamentinės parazitologijos mokslui Lietuvoje. Šiame straipsnyje aprašomi pagrindiniai dabartinių Gamtos tyrimų centro (Vilnius) P. B. Šivickio parazitologijos laboratorijos darbuotojų pasiekimai fundamentinėje parazitologijoje, ypač eukariotinių parazitų (protistų ir helmintų) molekulinį tyrimų srityje.

Raktažodžiai: P. B. Šivickio parazitologijos laboratorija, fundamentinė parazitologija, parazitų molekulinis ir morfologinis apibūdinimas