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Insect Trypanosomatids: the Need to Know More

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Of ten recognized trypanosomatid genera, only two – pathogenic Trypanosoma *and* Leishmania *– have been actively investigated for any length of time while the plant flagellates* – Phytomonas – *have recently begun to attract attention due to their role as agricultural parasites. The remaining genera that comprise parasites associated with insects have been largely neglected except for two or three containing popular isolates. This publication reviews current knowledge of trypanosomatids from insects.*

Key words: Trypanosomatidae - insect and plant trypanosomatids - evolution

The trypanosomatid evolutionary tree (Philippe 1998, Stevens et al. 1998, 1999) has in its base the monophyletic clade of *Trypanosoma*, while *Leishmania* is situated in the crown with a range of insect flagellate congeries. Interestingly, although *Trypanosoma* and *Leishmania* are superficially close to each other from a medical point of view, they are far from each another on the evolutionary tree, and many isolates from insects and plants are spread between *Trypanosoma* and the crown of the tree.

LEISHMANIA - SOME QUESTIONS

From a phylogenetic point of view *Leishmania* is no more than one of the insect trypanosomatid lineages which have successfully colonized two classes of terrestrial vertebrates. *Leishmania* representatives occur in abundance in mammals and reptiles, but only two doubtful cases of *Leishmania* infection have been reported in birds (Molyneux & Ashford 1983, Podlipaev 1990).

Birds serve as a host for various parasites, including many *Trypanosoma* species. *Leishmania* vectors – sand flies – can feed on birds in areas where *Leishmania* are abundant, for instance in Central Asia (Perfiliev 1966) and it is known that chickens are attractive for *Lutzomyia* sand flies in Brazil (Quinnell et al. 1992). Some data suggest that bird macrophages can kill *Leishmania in vitro*, but *Trypanosoma* parasites were also killed (Meirelles & De Souza 1985). The type of bloodmeal also affects the proteolitic activity of sand flies and subsequent *Leishmania* development (Daba et al. 1997), and it is very likely that more factors may arrest *Leishmania* development and/or growth in birds or in sand flies with birds blood in their intestine. However, any explanation has to elucidate the differences between parasites – the 'elder' *Trypanosoma* and the 'younger' *Leishmania* – which allow the former to successfully colonize birds while preventing the latter from colonising these hosts. Accordingly, it is important to elucidate the basis of the differences outlined above not only for academic reasons but in order to understand the mechanisms of vertebrate resistance to parasites and, finally, to help find new therapies for leishmaniasis and trypanosomiasis.

Interestingly, except for one obscure report of *Crithidia* sp. in *Phlebotomus* and experimental infection of *Phlebotomus* by *C. fasciculata* (see Podlipaev 1990) *Leishmania* vectors are free from their own monogenetic parasites. Obviously the lack of monogenetic trypanosomatids in sand flies needs to be checked; if the absence is corroborated, it may be a confirmation of the independent origin of digeneity in *Leishmania*.

INSECT TRYPANOSOMATIDS

Fauna and descriptions - During the first century of trypanosomatid studies only 350 species of insects have been identified as hosts of monoxenous trypanosomatids (Wallace et al. 1983, Podlipaev 1990). It can be estimated that from more than 1,000,000 known species of insects no more than 2,000-2,500 species have been studied by parasitologists. So, only a minority of insect taxa originating from a limited number of locations have until now been examined for the presence of trypanosomatids. The vast majority of insect trypanosomatids are still to be described and relationships among them and with *Trypanosoma* sp. need to be established. Exploration of new regions has brought many new findings; recently flagellates were found in a range of Hemiptera in Brazil

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(Sbravate et al. 1989), in two new insect families in Russia and their known distribution was extended to Central Asia, the Far East and north to the polar circle (Podlipaev 1990, Podlipaev unpublished observation). Paradoxically, at the end of the millenium extensive areas remain as *tabula rasa*: nothing or very little is known about insect trypanosomatids in Southeast Asia, Australia, Japan and many other areas.

Questions appear from the beginning – from species descriptions and nomenclature. Currently, insect trypanosomatids appear to have the greatest number of formally valid descriptions that cannot be used in research. The descriptions differ greatly from each other by their quality and often it is impossible to compare them; indeed, it is sometimes impossible to establish correspondence between an old description and a newly-found parasite (Podlipaev & Lobanov 1996). For example, from 69 described species of genus *Leptomonas* which could be estimated as formally valid taxa, only 15 may be viewed as reliable, with 10-15 descriptions being doubtful and the remaining 40 or so containing insufficient information to allow comparison with other findings (Podlipaev 1990).

A paradox occurs with the type species (by monotypy) of the genus *Leptomonas* – *L. buetshlii* Kent, 1880 from the nematode *Tobrilus* (*Trilobus*) *gracilis*. This report is the only finding of a trypanosomatid in a nematode and it has never been re-isolated. We also dissected numerous benthic nematodes, including *Trilobus* sp. and did not find any trypanosomatids (Podlipaev unpublished). Figures given in the published description do not allow this organism to be reliably assigned to the trypanosomatids or for it to be classified as a representative of another taxon. Latterly a flagellate parasitizing these nematodes has been shown to belong to Euglenidae (Nicoli et al. 1971), but it is impossible to be certain that it is the same organism as that originally described by Kent.

Distribution and host specificity - The important issue of host-specificity has often been treated somewhat arbitrarily. Such treatment ranges from the description of a new trypanosomatid from every insect host species to the assignment of parasites at the host family level (see Wallace 1966, Podlipaev 1990), finally resulting in the point of view that the majority of insect trypanosomatids are stenoxenous but that some of them may infect many hosts (Wallace et al. 1983).

Results obtained from DNA cross hybridization, multilocus isoenzyme electrophoresis, and random and universally primed polymerase chain reaction (PCR) indicate a very wide host specificity of insect trypanosomatids: no correspondence between natural groups of trypanosomatids (groups of distinctive genotypes) and host taxa were found. Genera, species or isolates of trypanosomatids from the insects do not coincide with species, genera and families of hosts and possibly do not even coincide with host orders. Insects from different families may have PCR-identical flagellates and parasites from hosts belonging to different orders may sometimes be genetically closer than trypanosomatids from the same host species (Podlipaev & Bulat 1998, Bulat et al. 1999).

The distribution of trypanosomatids in insects is very irregular; in two orders, Hemiptera and Diptera, about 300 described species and undetermined trypanosomatids have been reported. A few, sporadic or unreliable findings (about 20 descriptions in total) have been described in seven other orders (see Podlipaev 1990). Such disproportion is obviously determined by the evolution history of trypanosomatids and is widely discussed in the literature (Vickerman 1994). There are not enough data to judge whether co-evolution (or co-cladogenesis) of monogenetic trypanosomatids and hosts has taken place in the order Diptera. Within the Hemiptera the low level of host specificity provides evidence of the low level of co-evolution of monogenetic trypanosomatids and their hosts, and there is no correspondence between any group of insects trypanosomatids with hemipteran taxons at any level (Podlipaev & Bulat 1998, Bulat et al. 1999, Podlipaev & Rokitskaya 1999).

Similar conclusions may be drawn from faunistic data. For example, in the monophyletic group of semiaquatic bugs (suborder Gerromorpha) (Andersen 1981), water-striders (family Gerridae) are infected by trypanosomatids in abundance and universally (it appears probable that they are the most commonly infected of all insect taxa); for the family Veliidae only three (unconfirmed) reports are known and Hydrometridae are free of flagellates in all regions explored (Wallace 1966, Podlipaev 1985, 1990, 1999). The mode of distribution of trypanosomatids among Hemiptera indicates that the co-evolution of monoxenous parasites and their insect hosts appears unlikely. Of course, as only the minority of insect species have been investigated, this conclusion can only be regarded as preliminary.

CULTURES

In the last few decades investigation of trypanosomatids consists mostly of studies of trypanosomatid cultures. About 20 years ago 10-15 cultures (isolates) were used in various laboratories (Wallace et al. 1983); at present no more than 40 isolates are commonly under study. Such a limited number of isolates is clearly insufficient to characterize the diversity of insect trypanosomatids and is also probably insufficient to infer the true position of species in a phylogenetic tree.

Moreover, different cultures are investigated rather unequally. The 'favourite' and most well-studied are cultures of *C. fasciculata* and *C. oncopelti*. But, problems with these are apparent; since several *Crithidia* have been removed from the genus *Crithidia* to a monophyletic clade of symbiont bearing tryps (Du et al. 1994, Hollar et al. 1998), *C. fasciculata* is left as the only representative for the whole genus *Crithidia* on the rRNA tree. However, the figure from the original description of *C. fasciculata* L. Leger (1902) depicted epimastigotes among other cells – this fact casts some doubts on its identity and raises the possibility of mixed infection (Podlipaev 1990).

Similarly, there are several cultures designated as *C. oncopelti*, whose history is obscure. In one of them two independent cell populations that differ from each other by many features were discovered (Krylov et al. 1985). Latterly, it has been found that there are two isolates both named *C. oncopelti* differing from each other by the structure of rRNA genes (Du & Chang 1994, Clark 1997, Hollar et al. 1998).

A low level of specificity and the ability of trypanosomatids to survive in an alien host (Hanson et al. 1968, Carvalho & Deane 1974, Huppenrich et al. 1992) might enhance the probability of culturing mixed infections or a nonspecific (occasional) parasite. For example, from our data about 17% of findings in water-striders (Hemiptera: Gerridae) in Central Asia and the Caucasus are suspected to be mixed infections (Podlipaev unpublished).

Microscopic investigation of a host is far from being an ideal tool to determine the true nature of a mixed infection. For example, *Blastocrithidia gerricola* was identified as *Blastocrithidia* because of the presence of numerous (about 98%) epimastigotes in the host bug but, simultaneously, it was supposed, from the morphology of the cells in culture and from the presence of rare promastigotes in the host, that there was a mixed infection in the insect and that an organism other than *Blastocrithidia* might have been isolated (Podlipaev 1985). So, in the case of *B. gerricola* it appears that the minor component of the mixed infection was probably isolated instead of the 'main' parasite. Molecular markers showed that the *B. gerricola* culture belonged or was very close to another genus *Wallaceina* (Bulat et al. 1999).

On the basis of our collection, *a priori* we can be more or less sure that only two isolates represent a specific infection: *Leptomonas rigidus* from the bug *Salda littoralis*, because the host inhabits a very specific biotope (it lives under material washed ashore in the upper intertidal zone of the White Sea close to the polar circle) and is ecologically isolated from all other Hemiptera in the region (Podlipaev et al. 1991); and *Wallaceina brevicula*, which was isolated in the spring under snow during winter adult diapause (Frolov & Malysheva 1989).

Therefore, only the direct investigation of a culture may solve the question "what parasite was really isolated?". Of course, it must be remembered that the procedure of isolation itself and long laboratory cultivation may introduce some selective effect. If the data obtained from the morphological investigation of an original insect infection contradict those obtained from the study of a laboratory isolate it is necessary to consider the latter to determine the taxonomic position of the organism now in culture.

There is one more problem which is the opposite of that discussed above – this is the possibility of isolating representatives of different lifecycle stages in culture and then inadvertently mistaking these to be different taxa. Investigation of the *Wallaceina* lifecycle in the artificially infected insects demonstrated that in the host intestine the flagellates are represented by two morphologically different subpopulations that correspond to two 'strains' isolated from a laboratory culture (Malysheva & Frolov 1995). In the absence of direct experimental investigation these strains were taken to be separate parasite species (Frolov & Malysheva 1989); it now appears that these may in fact be different lifecycle stages of *Wallaceina brevicula* (Podlipaev et al. 1990).

HOST-PARASITE SYSTEMS

"Nature prefers that neither host nor parasite should be too hard on the other." M Burnet and DOWhite (1972), *Natural History of Infectious Disease*, p. 82.

Together, low level specificity, a high probability of nonspecific infection, and genetic and physiological variability of trypanosomatids may increase and facilitate the chances of establishing new host parasite systems. Moreover, it appears probably that not only insects but plants and other organisms may be involved in such interactions which can be more or less "occasional".

In some cases a paradox between the high level of parasite divergence in phylogenetic trees and the age of host-parasite systems which appear more recent is apparent. From published phylogenies (see Hollar & Maslov 1997, Philippe 1998, Stevens et al. 1998) it seems that the digenetic insect/plant clade – *Phytomonas* – also has its roots in insect trypanosomatids. Many Hemiptera, even predatory bugs, use plants sap as a source of fluid and it is possible that this may be a precondition to the nonspecific transfer of flagellates from insect to plant and between plants. Fruit provides a rich medium for the multiplication and growth of insect flagellates (Conchon et al. 1989) and it is not inconceivable that such numerous opportunities for transfer may eventually result in successful adaptation to development in carbohydrate-rich plant tissues.

The monophyletic *Phytomonas* clade appears on the basis of its position within the rRNA phylogeny to be recent (Hollar & Maslov 1997) and intraphloemic flagellates show considerable genetic differences from the latex parasites and from the parasites of insects (Muller at al. 1994, 1997, Bulat et al. 1999). On rRNA phylogenetic trees intraphloemic *Phytomonas* form the earliest branch of a monophyletic clade of plant flagellates (Marche et al. 1995, Hollar & Maslov 1997). Phloem inhabiting *Phytomonas* are the causative agents of epiphytotic plant diseases of introduced plants in South America – coffee, oil palm, coconut and the ornamental plant *Alpinia purpurata* (Dollett 1984, Camargo 1999). The damage caused by flagellates is so high that botanists consider *Phytomonas* to be a natural barrier for plant naturalization (Mack 1996); interestingly, coffee disease has reached epiphytotic proportions twice, in the beginning and in the middle of the last century (Camargo et al. 1990). Intraphloemic trypanosomatids have been found in four species of South American native palms (Camargo 1999), but no trypanosomatids have been found in coconut and oil palms in their native regions or in Southeast Asia where palm agriculture is very extensive.

Thus, from these data and from the classical 'rules' of parasitology such pathogenic, non-stable host-parasite systems appear somewhat new, arising, in the case of palms, within historical times.

The unpredictable and random mode by which trypanosomatids may enter a new host are illustrated by exotic cases of trypanosomatids infecting ciliate nuclei. The most well-studied is the finding of *Leptomonas ciliatorum* in the macronucleus of the gastrotrich *Paraholosticha sterkii* (Görtz & Dieckman 1987), where the symbiont is well-adapted to the host. Several other ciliates from various genera were experimentally infected by flagellates; in some cases attempts to infect ciliates succeded and in others it failed. Attempts to find infected ciliates in nature where they were found previously also proved negative.

The similarity in the origins of the "unexpected" host-parasite systems – intranuclear trypanosomatids/ciliates and *Phytomonas*/plant systems (e.g. flagellates/coffee) – is striking and the main features of host-parasite systems including the monogenetic trypanosomatids and *Phytomonas* are reflected in the cases mentioned above. I.e.: (1) occassionally 'choice' of the host, may be restricted to a particular genotype or clone (in a taxonomic sense – only a few representatives of any taxonomic level may serve as the hosts); (2) rapid co-adaptation to a particular host resulting in creation of an obligatory host-parasite system. Trypanosomatids indicate that a high level of co-adaptation does not always mean a long time period of co-evolution; (3) opportunist use of alternative hosts. Probably one such attempt resulted in the origination of *Leishmania* and plant trypanosomatids. And, it is possible, that recent reports about non-*Leishmania* and non-*Trypanosoma* parasites infecting humans (Dedet et al. 1995, Jimenes et al. 1997) fall into this category.

SYSTEMATICS

The taxonomic system of trypanosomatids is extremely conservative. From ten existing genera eight were described prior to 1909, one genus in 1959 and the last one in 1990 (see Podlipaev 1990).

The heterogeneity of *Crithidia*, *Leptomonas* and *Herpetomonas* have been demonstrated by different methods (Kolesnikov et al. 1990, Camargo et al. 1992, Du et al. 1994, Fernandes et al. 1997, Hollar et al. 1998, Bulat et al. 1999).

The monophyly of symbiont containing trypanosomatids (Du et al. 1994; Hollar et al. 1998) is the most impressive example of the unreliability of classical morphological criteria (Hoare & Wallace 1966). This clade includes *Blastocrithidia*, *Herpetomonas* and *Crithidia* species, each genus being clearly determined by cell morphology types. However, the existence of a symbiont bearing monophyletic clade clearly demonstrates that cell morphology is not appropriate as a taxonomic character for trypanosomatids and allows us to conclude that existing genera do not (and cannot) reflect the real biodiversity of trypano-somatids.

Insects trypanosomatids together with those genera possibly derived from them – *Phytomonas* and *Leishmania* – comprise a very polymorphic group. The existing ten genera clearly serve only to highlight the lack of reliable classification methods and, from another viewpoint, the lack of new isolates under study. Accordingly, current trypanosomatid systematics need to be revised. To gain a clear overview of trypanosomatid phylogeny we urgently need to increase our knowledge about the most variable group of trypanosomatids – the parasites of invertebrates and plants.

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