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THE SUB-GENERA OF AVIAN *PLASMODIUM*

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Summary:

The study of the morphology of a species of *Plasmodium* is difficult because these organisms have relatively few characters. The size of the schizont, for example, which is easy to assess is important at the specific level but is not always of great phylogenetic significance. Factors reflecting the parasite's metabolism provide more important evidence. Thus the position of the parasite within the host red cell (attachment to the host nucleus or its membrane, at one end or aligned with it) has been shown to be constant for a given species. Another structure of essential significance that is often ignored is a globule, usually refringent in nature, that was first decribed in *Plasmodium vaughani* Novy & MacNeal, 1904 and that we consider to be characteristic of the sub-genus *Novyella*. Species without this structure, previously classified in this sub-genus, are now included in the new sub-genus *Papernaia* n. sg.

KEY WORDS : Avian *Plasmodium*, sub-genera, *Novyella*, *Giovannolaia*, *Papernaia* n. sg.

Résumé : LES SOUS-GENRES DE *PLASMODIUM* AVIAIRE

La morphologie d'un Plasmodium *est difficile à étudier car on dispose de peu de caractères. La taille d'un schizonte, facile à apprécier, est significative au niveau spécifique mais n'a pas toujours une grande valeur phylogénique. Le métabolisme du parasite fournit des éléments plus importants. Ainsi, la situation du parasite à l'intérieur de l'hématie (accolé au noyau, ou à la membrane, au sommet ou le long du noyau) se révèle très constant chez chaque espèce. Un autre caractère, de valeur essentielle, trop souvent négligé, est le globule le plus souvent réfringent, décrit pour la première fois chez* Plasmodium vaughani *Novy & MacNeal, 1904 et que nous considérons comme caractéristique du sous-genre* Novyella. *Les espèces qui en sont dépourvues, précédemment classées dans ce sous-genre sont placées dans le nouveau sous-genre* Papernaia *n. sg.*

MOTS CLÉS : Plasmodium *aviaire, sous-genres,* Novyella, Giovannolaia, Papernaia *n. sg.*

Many contemporary writers, impressed by the data ensuing from the process of molecular typing, appear to consider that morphological data can be ignored. We believe, however, that the latter are indispensable, not only in terms of nomenclature, but also for an understanding of the grouping of organisms. This paper deals with the example of the genus *Plasmodium* in birds.

THE FREQUENCY OF CONGENERIC PARASITE SPECIES

The phenomenon of multiple parasite infections in a single host is one that has been observed in a number of parasite groups. Some examples are the flagellate protozoa of termites (Grassé, 1926), ciliates of the rumen of cattle (Noirot-Thimotee, 1960), *Isospora* of sparrows (Grulet *et al.*, 1982, 1986), *Eimeria* of rabbits (Coudert, 1995) and hares (Aoutil *et al.*, 2005), *Plasmodium* of Man (Garnham, 1966), oxyurids of terrestrial tortoises (Petter, 1966) and trichostrongylids of numerous vertebrates (Durette-Desset, 1969). This phenomenon was explored in depth by Chabaud & Durette-Desset (1978) who gave a detailed account of the concepts of both stable and unstable equilibrium in parasitic populations. We have observed the phenomenon again very clearly in relation to the *Plasmodium* of birds, both magpies (*Pica pica*) in the region of Paris (14 species identified) and skylarks (*Alauda arvensis*) captured in the Landes during the course of their autumnal migration (14 species) (Chavatte *et al.*, 2007, 2008).

It seems very likely that a primitive species of monoxenous parasite would have the possibility of becoming isolated and undergoing specific changes resulting from, for example, localisation in a slightly different host or location in a distinctive ecological niche. Such possibilities for diversification by isolation, followed by speciation would naturally be more numerous in the case of heteroxenous parasites.

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MORPHOLOGICAL DATA

The above phenomenon has been identified and described especially in two groups of parasites, the nematodes and the sporozoa. In the former the process of parasite evolution is relatively simple to follow since the free ancestors, the rhabditic helminths, are known and their morphological characters are very abundant. In the case of the sporozoa the morphology is infinitely more difficult to interpret. It is essential to attach much importance to each morphological modification that may be associated with the parasite's metabolism. A parasite that is adherent to the nucleus of a host's haematocyte is different from one that is attached to the host cell membrane or that lies free in the host cell cytoplasm. A parasite that destroys the nucleus or in other way modifies the haematocyte host differs from one that leaves the host cell intact. Thus we consider the presence or absence of a refractory globule such as that in *P. vaughani* Novy & MacNeal, 1904 to be as important as the presence or absence of pigment as in the *Garniidae* (Lainson, Landau & Shaw, 1971) which are indicative of specific types of metabolism in these organisms.

MOLECULAR AND MORPHOLOGICAL ANALYSIS

In a recent and very interesting publication on the *Plasmodium* of birds, Martinsen *et al.* (2006) compared the value and reliability of the molecular characterisation of species to the morphological analysis. Our own conclusions differ from theirs for the reasons that follow.

POLYPARASITISM AND SPECIES IDENTIFICATION

In the best analysed examples of multispecies infection in a single host a true equilibrium has been shown to become established. Certain parasite species are present in abundance while others are present but in much smaller numbers. In the case of avian *Plasmodium* the latter parasites can be extremely difficult to visualise on blood film preparations. Also, too often a specific identification is uncertain because of the paucity of the infection or the absence of morphologically characteristic stages. On the other hand, in very heavy infections the morphology of the parasites may be modified and untypical, or the abundance of one species may mask the presence of others. Finally it is not uncommon, when sub-inoculations are made from the natural to experimental hosts, that species not recognised in the former appear in the latter where, in blood films, they can be falsely interpreted as examples of the original parasite species that have undergone morphological changes in the experimental host. We almost never encounter monospecific infections and find it difficult to understand how it is possible to attribute a particular molecular sequence to a mixture of species, one or more of which are cohabiting often at an extremely low level.

PLACE OF MOLECULAR ANALYSIS AND PARASITE PHYLOGENY

The use of molecular analysis is able to yield precise indications of the phylogeny of species but it is incorrect to state that *Plasmodium* morphology is not able to do this. The example quoted below that is intended to demonstrate how morphology has little value, in fact points in exactly the opposite direction. "For example, *Plasmodium giganteum* Theiler, 1930 a parasite of west African lizards, that produces enormous schizonts in erythrocytes containing ~ 100 merozoites, is not related to other giants *Plasmodium*, but is the closest sister to the relatively tiny *Plasmodium agamae* Perkins & Schall, 2002" (Martinsen *et al.*, 2007). Garnham in 1966 had already written: "*P. giganteum* is clearly related to *P. agamae...*"

We have emphasised the fact that, while very similar species of *Plasmodium* coexist in the same host, they not only occupy different spatial or temporal niches (Cambie *et al.*, 1990) but also have different sizes. In this respect, the similarities between the *Plasmodium* of lemurs and of rodents were pointed out by Landau *et al.*, (1989). *Lemur macaco* hosts three related species of *Plasmodium*: *Plasmodium bucki* large (32 merozoites in the mature schizont), *Plasmodium percygarnhami* of medium size (20 merozoites), *Plasmodium coulangesi,* small (six merozoites). In the rodent *Thamnomys rutilans*, three close species of different size were found: *Plasmodium yoelii* is the large species, *Plasmodium chabaudi* the smallest and *Plasmodium vinckei* of intermediate size.
The size of schizonts, even if this allows one to differentiate one species from another, has little value at the generic level.

ANALYSIS OF SUB-GENERA

In 1963 Corradetti, Garnham & Laird proposed a new classification of the avian malaria parasites which they divided into four sub-genera (*Haemamoeba, Giovannolaia, Huffia* and *Novyella*) based on the size of the schizonts, the form of the gametocytes and the type of exo-erythrocytic schizogony. In 1997, Valkiũnas created a new sub-genus (*Bennettinia*) for a very unusual parasite of galliforms: *Plasmodium juxtanucleare* Versiani & Gomes, 1941. An analysis of the

Sub-genus	Gametocytes	Globule	Displacement of host-cell nucleus	Position of schizonts	Host cells
Haemamoeba	Round	–	Frequent	Variable	RBC
Bennettina	Round	–	None	Adherent to host cell nucleus	RBC
Huffia	Elongated	–	Frequent	Variable	All cells of the blood
Novyella	Elongated	+	None	Polar or latero-polar	RBC
Giovannolaia	Elongated	–	Sometimes laterally	Longitudinal	RBC
Papernaia	Elongated	–	Sometimes tilted	Polar or latero-polar	RBC

Table I. – Main distinctive characteristics of avian *Plasmodium* sub-genera. RBC = red blood cell.

criteria utilised allows as a first step the separation of sub-genera into two groups: species with round gametocytes (*Haemamoeba, Bennettinia*) and species with elongated gametocytes (*Giovanniola, Huffia* and *Novyella*) (Table I).

The work of Martinsen *et al.* (2007) with two mitochondrial genes validated three of the five sub-genera (*Haemamoeba, Bennettinia, Huffia*) and agreed on their monophyly.

The species attributed to the sub-genus *Novyella* did not form a monophyletic group since two of the taxa separated as an isolated clade distinct from the other "*Novyella*" and from all the other sub-genera. Moreover, the species classified in the sub-genus *Giovannolaia* form a polyphyletic group since several of the sequences revealed are also found among those of other sub-genera.

We have, therefore, analysed the species attributed to these two sub-genera from a purely morphological point of view.

NOVYELLA

Parasites in the sub-genus *Novyella* were defined by Corradetti *et al.* (1963) as having small schizonts, very little cytoplasm, elongated schizonts and exo-erythrocytic schizogony in the reticuloendothelial system but not in erythroblasts. The type species is *Plasmodium vaughani* Novy & MacNeal, 1904, described from *Turdus migratorius* Linnaeus, 1766, in Michigan.

A morphological feature that we believe to be essential, present in the type species and in most of the other species included in the sub-genus *Novyella*, but absent from those in other sub-genera, is a globule, usually refractory, white, pale blue or a clear blue, that is seen in the intraerythrocytic stages of the developing parasites. It remains unchanged through the whole development of the parasite and as a residue when the schizont matures. While its nature and role remain unknown, transmission electron microscope studies (Chavatte *et al.*, submitted) showed that it is totally different from transparent vacuoles that are frequently seen in blood smears of other species of *Plasmodium.*

We therefore propose to amend the definition of the sub-genus *Novyella* to include: presence in the cytoplasm of one or more usually refractory "vitreous"globule. Since the characters of the type species determine the definition of the genus or sub-genus, we therefore are led to remove from this sub-genus the following species that have been classified with *Novyella* but that lack the refractory globule: *Plasmodium bertii* Gabaldon & Ulloa, 1981; *Plasmodium columbae* Carini, 1912; *Plasmodium rouxi* Sergent, Sergent & Catanei, 1928; *Plasmodium hexamerium* Huff, 1935; *Plasmodium nucleophilum* Manwell, 1935; *Plasmodium paranucleophilum* Manwell & Sessler, 1971; *Plasmodium ashfordi* Valkiũnas *et al*., 2007 and *Plasmodium lucens* Valkiũnas *et al*., 2009.

Plasmodium nucleophilum toucani Manwell & Sessler, 1971, may differ from the last named species and belong to the sub-genus *Novyella* if it is confirmed that the small blue vacuole reported by the authors is indeed a globule.

Thus the sub-genus *Novyella* contains at present: *P. vaughani*; *Plasmodium tenuis* (Laveran & Marullaz, 1914); *Plasmodium merulae* Corradetti & Scanga, 1972; *Plasmodium kempi* Christensen, Barnes & Rowley, 1983; *Plasmodium dissanaike* De Jong, 1971; *Plasmodium mohammedi* Paperna *et al*., 2008, *Plasmodium pachysomum* Paperna *et al.,* 2008, *Plasmodium stellatum* Paperna *et al.,* 2008, *Plasmodium multivacuolaris* Valkiũnas *et al*., 2009; *Plasmodium parahexamerium* Valkiũnas *et al*., 2009; *Plasmodium globularis* Valkiũnas *et al*., 2008; *Plasmodium megaglobularis* Valkiũnas *et al*., 2008 and *Plasmodium accipiteris* Paperna *et al*., 2007 (Table II)

GIOVANNOLAIA

This sub-genus was defined by Corradetti *et al.* (1963) as having large schizonts, abundant cytoplasm, elongated gametocytes and exo-erythrocytic schizogony in the reticuloendothelial system. The type species is *Plasmodium circumflexum* Kikuth, 1931. The authors draw attention, however, to the fact that certain species are difficult to classify within one or other of the last two sub-genera and give, as an example, the case of *Plasmodium polare* Manwell, 1934 which is relatively small, produces on the average nine merozoites and has abundant cytoplasm.

Sub-genus	Species	References
Novyella	*accipiteris*	Paperna & Landau, 2007
	dissanaike	De Jong, 1971
	globularis	Valkiūnas *et al.*, 2008
	kempi	Christensen *et al.*, 1983
	megaglobularis	Valkiūnas *et al.*, 2008
	merulae	Corradetti & Scanga, 1972
	mohammedi	Paperna *et al.*, 2008
	multivacuolaris	Valkiūnas *et al.*, 2009
	nucleophilum	
	toucani	Manwell & Sessler, 1971
	pachysomum	Paperna *et al.*, 2008
	parahexamerium	Valkiūnas *et al.*, 2009
	stellatum	Paperna *et al.*, 2008
	tenuis	(Laveran & Marullaz, 1914)
	vaughani	Novy & McNeal, 1904
Giovannolaia	*anasum*	Manwell & Kuntz, 1965
	buteonis	Paperna & Landau, 2007
	circumflexum	Kikuth, 1931
	fallax	Schwetz, 1930
	ghadiriani	Chavatte & Landau, 2007
	gundersi	(Bray, 1962)
	heroni	Basu, 1938
	lophurae	Coggeshall, 1938
	octamerium	Manwell, 1968
	tranieri	Chavatte & Landau, 2007
Papernaia	*ashfordi*	Valkiūnas *et al.*, 2007
	beaucournui	Chavatte & Landau, 2007
	bertii	Gabaldon & Ulloa, 1981
	columbae	Carini, 1912
	dherteae	Chavatte & Landau, 2007
	durae	Herman, 1941
	formosanum	Manwell, 1962
	gabaldoni	Garnham, 1977
	garnhami	Guindy *et al.*, 1965
	golvani	Chavatte & Landau, 2007
	hegneri	Manwell & Kuntz, 1966
	hexamerium	Huff, 1935
	jeanriouxi	Chavatte & Landau, 2009
	lenoblei	Chavatte & Landau, 2007
	nucleophilum	
	nucleophilum	Manwell, 1935
	paranucleophilum	Manwell & Sessler, 1971
	pediocetae	Shillinger, 1942
	pinotti	Muniz & Soares, 1954
	polare	Manwell, 1934
	reniai	Chavatte & Landau, 2009
	rouxi	Sergent *et al.*, 1928
	snounoui	Chavatte & Landau, 2007
	valkiunasi	Chavatte & Landau, 2007

Table II. – Checklist of *Plasmodium* sub-genera and assigned species.

Since this sub-genus was described it has become a sort of general pot into which have been dropped all the species that have not been classified elsewhere. This situation is reflected in the work of Martinsen *et al.* (2007): the three samples identified as belonging to the *Giovannolaia* sub-genus "fell within three distant clades and clearly did not form a monophyletic group". The round or elongate structure of the gametocytes differentiates two groups of parasites: *Haemamoeba* and *Benettina* in one, *Giovannolaia*, *Novyella* and *Huffia* in the other. We believe and molecular analysis indicates that the sub-genus *Giovannolaia* as previously employed contains species of a disparate nature. We propose here that only species in which the principal morphological characters correspond to those of the type species, *i.e. P. circumflexum*, in which gametocytes and schizonts are stretched along the red cell nucleus, should be retained in this sub-genus: *P. circumflexum* Kikuth; *Plasmodium fallax* Schwetz, 1930; *Plasmodium lophurae* Coggeshall, 1938; *Plasmodium ghadiriani* Chavatte & Landau, 2007; *Plasmodium anasum* Manwell & Kuntz, 1965; *Plasmodium tranieri* Chavatte & Landau, 2007; *Plasmodium gundersi* (Bray, 1962), *Plasmodium octamerium* Manwell, 1968; *Plasmodium heroni* Basu, 1938 and *Plasmodium buteonis* Paperna *et al.*, 2007 (Table II).

The remaining group of parasites having elongated gametocytes has schizonts which are rounded or with an irregular shape, apically or latero-apically placed: *Plasmodium hegneri* Manwell & Kuntz, 1966, *Plasmodium gabaldoni* Garnham, 1977; *Plasmodium golvani* Chavatte & Landau, 2007; *Plasmodium reniai* Chavatte & Landau, 2009; *Plasmodium polare*; *Plasmodium durae* Herman, 1941; *Plasmodium pediocetae* Shillinger, 1942; *Plasmodium valkiunasi* Chavatte & Landau, 2007; *Plasmodium dherteae* Chavatte & Landau, 2007; *Plasmodium pinotti* Muniz & Soares, 1954; *Plasmodium formosanum* Manwell, 1962; *Plasmodium garnhami* Guinday, Hoogstraal & Mohammed, 1965; *Plasmodium lenoblei* Chavatte & Landau, 2007; *Plasmodium beaucournui* Chavatte & Landau, 2007; *Plasmodium snounoui* Chavatte & Landau, 2007 and *Plasmodium jeanriouxi* Chavatte & Landau, 2009. Others because of their small size were previously classified into the sub-genus *Novyella*: *P. hexamerium*, *P. columbae*, *P. rouxi*, *P. bertii*, *P. ashfordi*, *Plasmodium nucleophilum nucleophilum*, *Plasmodium paranucleophilum* Manwell & Sessler, 1971 (Table II). We propose to group these into a new sub-genus: *Papernaia* in honour of the late Ilan Paperna who contributed so much to our understanding of the morphology and the biology of many groups of parasites and to the taxonomy of bird malaria.

Definition of this sub-genus: *Plasmodium* with elongate gametocytes, schizonts apically or lateroapically placed, rounded or irregularly shaped. Type species: *Plasmodium polare* Manwell, 1934 in the cliff swallow *Petrochelidon pyrrhonota* (Vieillot, 1817) in Syracuse (USA). The main characteristics of the sub-genera are presented in the Table I.

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