THE PROBLEM OF SPECIES IN *Novyella*

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The proper criteria of species has always been a problem in malariology, as indeed it is in biology generally. This is in part because the usual definition of species —that of a group of organisms closely resembling one another morphologically, and interbreeding freely among themselves but not with members of other groups is not easily applied to most protozoa, including *Plasmodium*, and in part for reasons of practicality and convenience.

Although interbreeding of different species of *Plasmodium* in the gut of a susceptible mosquito, previously fed on a vertebrate host with a mixed infection (and such infections are common enough in nature), is at least possible, it is a possibility which has seldom been taken into account by malariologists and nothing is known as to whether such hybridizing ever occurs. It is a problem worth laboratory study, as the author pointed out many years ago (3), but so far no one seems to have attempted it.

From a purely practical point of view, species identification of malaria parasites must usually be made from blood films, and therefore other characteristics, equally important, such as the possibly differing morphology of exoerythrocytic stages, the location and duration of such infection in the host, the limits of natural variation, virulence in natural and laboratory hosts, host specificity, and the like cannot be utilized. For many species little is known about such matters anyway.

Among the avian malaria parasites this is especially true of the species belonging to the somewhat ill-defined subgenus *Novyella*. Although such species are not the commonest cause of malaria in birds and —taken as a group— probably not the most virulent and hence perhaps of little importance in nature, they occur throughout the world. They are also of interest because several species in the group parallel closely certain reptilian plasmodia.

Garnham (1) defines *Novyella* as an assemblage of avian malaria parasites in which the erythrocytic merozoites consist mainly of chromatin and the gametocytes are elongate. All erythrocytic stages are relatively small. He also states that segmenters produce no more than eight merozoites.

The species included in the subgenus he lists as five: *Plasmodium vaughani*, *P. rouxi*, *P. hexamerium, P. nucleophilum*, and *P. juxtanucleare*. He also remarks, "the correct placing of a few of these parasites is slightly difficult, because they do not fit exactly into any subgenus as defined here . . .". It is this fact, plus difficulties presented in the definition of what seem new species, which forms the basis of this paper.

These species may be further divided into two groups, depending on host specificity. The first four infect mainly or entirely passerines, at least in nature, whereas *P. juxtanucleare* occurs only in gallinaceous species. However ducklings are also susceptible to infection with *P. hexamerium* and *P. nucleophilum*, when intravenous inoculation of infected blood is done. Whether they could be infected by sporozoites of these two species has never been determined.

There are also clear-cut differences in morphology separating the five species, though these are not so definite that recognition of mixed infections would always be easy, and they do not apply equally to all stages. *Plasmodium nucleophilum* usually adheres to the host cell nucleus, though this is not invariable. So doe's *P. juxtanucleare*, but most stages (including the often oval gametocytes) are fairly easily distinguishable from similar stages of *P. nucleophilum*, and it is not known to infect passerines. However this is a matter which should be investigated further. I have occasionally seen parasites in wild-caught imported passerines which could have easily been mistaken for *P. juxtanucleare*.

The other three species are more alike. All three are small and overlap in the size of merozoite broods, the progeny ranging from four to eight. It is always four in the case of *P. rouxi*, usually four for *P. vaughani* (though often as many as eight), and six for *P. hexamerium* (hence the name, but the range here is also from four to eight).

Among other characteristics exhibited by these species which are of use in identification are (1) the so-called "bow-tie" appearance often assumed by the more nearly mature schizonts of *P. rouxi* (a peculiarity first pointed out by Mohammed (6), (2) the refractile granule usually seen in the larger trophozoites and segmenters *of P. vaughani*, and (3) the oblique polar position in the erythrocyte frequently taken by the larger trophozoites of *P. hexamerium*. Although there have been reports of vaughani malaria in which no retractile granules were seen in the parasites, it seems to me that their absence makes identification as such very questionable. It is also clear that trophozoites of species other than *P. hexamerium* may assume an oblique position at the polar end of the parasitized erythrocyte, but much less commonly. Taken with other characteristics it is useful in diagnosis.

Gametocytes of all three species are elongate, lying beside the host cell nucleus which they may displace a little laterally, and with a few conspicuous pigment granules. They differ very little in morphology.

The host cells for all three are erythrocytes, usually mature, and are not noticeably altered.

Not only do morphological resemblances indicate a close relationship of these five species, but their behavior in the host is confirming evidence. Both in wild birds in which the infection was naturally acquired and in blood-induced laboratory infections there is no well-marked acute stage, and a moderately low-grade parasitemia usually persists more or less indefinitely. Sometimes a little search may be necessary before a parasite is found, but more often they are quite easily spotted. For this reason these malarias are much easier to detect by blood examination than are chronic infections caused by parasites of the larger species.

Host specificity is a debatable subject in the field of avian malaria. Although no one still believes that a new host record means a new species of malaria parasite, and it has been known for a long time that there is less host specificity among the avian malarial than in perhaps any other group of parasites, it does exist. Birds of some orders, such as the psittacines, have very seldom been found infected, and in my judgement, some such reports are questionable. We have attempted infection of Budgerigars by the inoculation of blood carrying malaria parasites of one or another of a variety of species without success, even though the recipient birds had previously undergone splenectomy and/or cortisone treatment to reduce natural resistance.

In birds of other orders, such as the Columbiformes, malaria seems uncommon. When it occurs reports in the literature indicate that it is usually due to some strain of *P. relictium*. I have examined hundreds of pigeons in the Syracuse area with wholly negative results as far as malaria is concerned.

Yet host specificity, at least of a relative sort, seems to be a fact with some species of the *Novyella* group. *Plasmodium vaughani*, as already stated, is extremely common in North American robins, and quite uncommon or unknown in other passerines. For example, we have never found it in any species of sparrow or blackbird, though the total number of individuals examined runs into hundreds. Nor have we have ever seen it in the English sparrow (actually a weaver bird, and not a sparrow), though well over a thousand have been examined.

Plasmodium juxtanucleare may be an even better example. It is thought to occur naturally only in gallinaceous birds. The chicken, from which it was originally described, is quite certainly not the natural host. The latter may well have been jungle fowl, which have been found infected, or partridges. In Formosa the Bamboo partridge (*Bambusicola thoracica sonorivox*) is a natural reservoir (4).

When considering host specificity of any species of malaria it is of course necessary to consider the invertebrate as well as the vertebrate host. But so far little is known about mosquito transmission of any members of *Novyella*. Partial exceptions are *P. hexamerium* and *P. juxtanucleare*. It is not even certain that mosquitoes are always the vectors.

What we may call the *Novyella* problem is illustrated both by *Plasmodium nucleophilum* and *P. polare* The former may produce as many as nine merozoites per segmenter (though usually no more than eight), and *Novyella* is, by definition, a group in which there is a maximum of eight. *Plasmodium polare*, on the other hand, develops segmenters with as few as eight merozoites of relatively small size, which might seem to put it in the subgenus of *Novyella* except that the mean size of a brood is 10.5. Gametocytes are elongate, but larger than those of most species consigned to *Novyella*. Garnham, who places it in the subgenus *Giovannolaia* nevertheless concedes that it lies "nearer to the *rouxi-hexamerium* than the *fallax-circumflexum* groups".

Plasmodium octamerium is a somewhat similar example. About 80 per cent of its segmenters produce eight merozoites, although the range is from six to twelve in the canary and larger numbers have been seen in the tree sparrow. Merozoites are also quite small, with scanty cytoplasm, and gametocytes elongate (though larger than

those of most species of *Novyella*). Garnham's comment about *P. polare*, quoted in the preceding paragraph, might also be made about *P. octamerium*, but the relatively large size of the sexual forms and the kind of cells parasitized by exoerythrocytic stages (in this case, not those of the lymphoid-macrophage system) seem to preclude placing it in *Novyella*, and it has instead been consigned to *Giovannolaia* (5).

Plasmodium tenue, described by Laveran and Marullaz in 1914 (2) and in limbo most of the time since because of the brevity of their description is another case in point. It was apparently seen by no one except Wenyon (7) In the years intervening until it was found in my laboratory in a babbler (*Liothrix luteus*) recently, the same host species in which it was originally observed. This babbler is a native of China and northern India, and is known to the pet trade as a Pekin or Chinese robin, or sometimes as a Japanese nightingale. Babblers, of course, are neither robins nor nightingales. They belong to the Timaliidae, and live in the Old World tropics. Since Wenyon also saw the parasite in the same host it seems rather likely there is some host specificity.

Wenyon's observations were made in a letter to the Sergent brothers and Catanei (7), who had recently discovered *P. rouxi*. As a result of a careful comparison he came to the conclusion that the two species were indeed distinct, though morphologically very similar, largely because *P. rouxi* invariably gives rise to four, and only four merozoites, while *P. tenue* may produce as many as six, as indicated in the table below, (based on our own observations). It clearly belongs in *Novyella*.

Number per segmenter	2	3	4	5	6	Total
Number counted	2	11	141	17	29	200
Percentage	1	5.5	70.5	8.5	14.5	100%

Table 1. Merozoite numbers produced by *Plasmodium tenue*.

There is the additional difference that canaries seem immune to infection by *P. tenue*, at least when the route of infection is intravenous and the inoculum is blood from infected babblers. Whether infection would result from the bites of infected mosquitoes is unknown, but seems rather unikely

All this raises the question of what the proper criteria of species should be in this group. Essentially it is a matter of the genetics of members of the group, and how much variation it is permissible to have within a species. All species of *Novyella* are small. The asexual stages are similar enough to make a mixed infection very difficult to identify in many cases. Subinoculation into a laboratory host, such as a canary, may not help very much.

Gametocytes, except those of *P. juxtanucleare* and *P. nucleophilum*, are very much alike, and the latter differ chiefly in their propensity to adhere to the host cell nucleus. Merozoite broods, except those of *P. rouxi*, vary within a nearly similar range, usually four to eight, though there are occasionally more and sometimes less (as in the cases of *P. tenue*, *nucleophilum*, and the so far unnamed species from the Silver-beaked tanager mentioned below).

The question becomes of real importance when one encounters malaria parasites which obviously belong to *Novyella*, but which differ from the other small species in characteristics such as merozoite number, though having similar gametocytes. We recently found infections in two Silver-beaked tanagers (*Ramphocelus carbo*). These birds were part of a batch of tanagers of several species received from a dealer who had imported them from Colombia. It seems at least possible that the parasites had some host-specificity, since no other tanagers in the batch apparently harbored the infection, nor did tanagers of a number of other species which were received from the same source later. The question of host specificity can only be answered by the examination of more Silver-beaks and, of course, birds of this and other families from the same area. So far we have been unable to secure more of the former.

Counts of progeny of the Silver-beaks' parasites, which were numerous on the slides, showed that about one-third of the broods numbered only two, though the majority produced four and occasionally as many as six. Although the table below if based on a count of 600 segmenters, in which the maximum number of merozoites was six, one brood of eight was later observed. This either means that occasionally more than six merozoites are normally produced or, just possibly, a mixed infection. Without infections in laboratory hosts, produced by subinoculation or the bites of infected mosquitoes, there is obviously no way of knowing which it was.

Merozoite number	2	3	4	5	6	Totals

Number counted	200	76	283	13	28	600
Percentage	33	14	47	2	4	100%

Table 2. Brood counts in Silver-beak strain of *Novyella*.

Does a marked difference in the number of merozoites per segmenter justify the designation of a new species, when other morphological differences are slight? Wenyon (7) thought it did when *P. rouxi* and *P. tenue* were involved, although it is fair to add that in this case, since *P. rouxi* always produces four merozoites per segmenter, brood size is definite rather than variable. One can partially evade the problem by calling a strain of this sort a subspecies, or evade it further by terming it simply a variant strain of *Novyella*, species undetermined. But neither solution seems satisfactory.

In the case of the Silver-beak strain, laboratory infections were attempted in canaries, but without success. The primary difficulty was that each of the tanagers had a mixed infection. The second species of the mix (*relictum* in one case and *circumflexum* in the other) killed each of the recipient canaries before enough time had elapsed for the *Novyella* strain to develop, assuming that the canary would have proved a susceptible host. Consequently the additional information which laboratory infections might have furnished remains lacking.

Exoerythrocytic stages were sought in the tanagers, which died of unknown causes several weeks after arrival, but without success.

Thus we are left with an unsolved problem. Here we have a strain of *Novyella* in which a large proportion of segmenters produce only two merozoites, a characteristic of no other known species, but which in most other respects differs rather little from other species of the subgenus. If we give it species or subspecies rank and a name there will be a record in the literature of its existence, but the objection may be raised that it would be better simply to regard it as a variant within the group. In the latter case there will be no specific record of its discovery in the literature.

It is of course true that the literature is filled with descriptions of species which have later had to be sunk because more knowledge proved them synonyms, yet if they were accurate to the extent that the material on which they were based permitted they should be regarded as necessary steps in the growth of a field, and hence real contributions.

What should we regard as the minimum criteria of a species of *Plasmodium*? It is a question which becomes progressively more difficult as the number of known species increases. As a practical matter, we must depend on stained blood films for identification and comparison of Haemosporidia, and therefore the asexual stages and gametocytes of a species should differ enough from corresponding stages of other species to be easily recognizable. But this is an ideal not realized for a number of species generally regarded as valid, e.g. *Plasmodium relictum* and *P. cathemerium* With some of the species in the *Novyella* complex, we are even farther from its realization. The amount of overlap between *P. rouxi*, *P. vaughani*, *P. hexamerium*, and *P. tenue* is considerable.

The first three of these four species have been generally regarded as valid for many years now, but it is possible that had they all been available for study before any of them were given names they would have been regarded as subspecies rather than species. The creation of subspecies however solves no problems; the criteria of subspecies are even more difficult to lay down than those of species. But the distinction between subspecies need be less sharp than between species, and more account can be taken of natural variation. Host specificity, geography, differences in virulence and physiology, and the like, may become important.

Other characteristics not to be overlooked in the description of species and subspecies are those involving the exoerythrocytic cycle, species acting as vectors and behavior of the parasites in vectors, and host distribution. But such knowledge is seldom available when species of *Plasmodium* are first discovered.

In summary, it may be said that *Novyella* is a complex of closely related species, most of them much more similar than species in other subgenera of avian *Plasmodium*. Even the subgenus is not well defined.

Recognition and identification of species of *Novyella* is difficult, sometimes even for one who knows the group quite well. This is especially true of mixed infections, which are common. It is quite probable that some reports in the literature of the finding of these species are cases of misidentification. This is because gametocytes (except for

those of *P. juxtanucleare* and *P. nucleophilum*) are similar, merozoite numbers per brood overlap (with four being a usual count), and the smaller asexual stages may be almost indistinguishable. Host specificity of a limited sort may exist, but many passerine species may harbor species of *Novyella* (*P. juxtanucleare* being, of course, an exception).

There is probably no group of species of avian malaria parasites about which so little is known. This extends to life cycles, transmission, behavior in the vector (in most cases unknown), host distribution, and the limits of natural variation. There is even disagreement concerning what should be the proper criteria of species.

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