Herpetology Notes, volume 16: 669-672 (2023) (published online on 10 September 2023)

Unusual double erythrocyte infection by heterogeneric parasites in the lacertid lizard *Psammodromus algirus* (Linnaeus, 1758)

Sara Blázquez-Castro[1,*], Rafael Barrientos[2], and Rodrigo Megía-Palma[1,3]

The term "multiple infection" describes the co-occurrence of multiple parasites of the same genus (i.e., monogeneric) in a single host cell (Martínez-de la Puente et al*.,* 2006). This phenomenon can occur both in erythrocytes and leukocytes of the hosts. For example, up to six sporozoites of *Schellackia* (Apicomplexa: Coccidia: Eimeriorina) were found to infect a leukocyte in a blood smear of Asian grass lizard, *Takydromus sexlineatus* Daudin, 1802 (Megía-Palma et al., 2014). On the other hand, "double infection" specifically describes the co-occurrence of two monogeneric parasites in a single blood cell (Jovani et al., 2004). In the case of parasites with sexual stages (gametocytes), observations of double gametocyte infections have been documented for the parasite genera *Plasmodium* (in reptile hosts), *Haemoproteus* (in both avian and reptile hosts), *Leucocytozoon* (in avian hosts), *Karyolysus* (in reptile hosts), *Hepatozoon* (in both avian and reptile hosts), and other hemogregarines (in reptile hosts), and also with *Schellackia* sporozoites (in reptile hosts) (Aparicio and Cordero del Campillo, 1980; Mutinga and Dipeolu, 1989; Jovani et al., 2004; Roca and Galdón, 2010; Shafeey et al., 2019). In fact, double infections represent relatively common observations in avian and reptile hosts (e.g., Jovani, 2002; Jovani et al., 2004; Jovani and Sol, 2005; Martínez-de la Puente et al., 2006; Megía-Palma et al., 2014).

Multiple hypotheses have been proposed to explain this phenomenon. For example, double infections may favour parasite transmission between hosts because their cells are more susceptible to a founder infection (Jovani, 2002). Also, they can be adaptive for gametocytes in the host blood if double infections increase the encounter rate between male and female gametocytes (Jovani, 2005; Martínez-de la Puente et al., 2006), which might be crucial when their densities in the host are relatively low (Jovani, 2002). However, the evidence supporting the latter hypothesis has been mixed. For example, a study in parasites of the genus *Plasmodium* demonstrated that double infection frequency was independent of parasite density (Jovani and Sol, 2005), but other studies found a positive relation between this and the presence of multiple infections (Martínez-de la Puente et al., 2007; Martínez-de la Puente and Merino, 2008).

Alternatively, other authors have proposed that multiple infections could be induced by host-related factors (Miller et al., 1984; Franzén et al., 1989; Ramasamy et al., 1999; Martínez-de la Puente et al., 2007). For example, Miller et al. (1984) and Franzén et al. (1989) found that the presence of monoclonal antibodies increased the number of multiple infections in *Plasmodium*. This might be adaptive for the host because parasite gametocytes find it difficult to reach maturity in multiple infected cells (Ahmed and Mohammed, 1978; Inselburg, 1983; Martínez-de la Puente et al. 2007). Therefore, according to the host adaptive hypothesis (Martínez-de la Puente et al., 2007), a high number of multiple infections may benefit hosts by reducing the total number of infected cells (e.g., Miller et al., 1984). This can be achieved by a more efficient immune response that would kill more parasites in fewer host cells. In turn, a milder anaemia will be produced in those infections that demanded destroying fewer host cells (Martínez-de la Puente et al., 2007). Nevertheless, double infections can also occur randomly, with no adaptive function for the host nor the parasite, because parasites undergo merogony, or another type of asexual reproduction, in host blood

[1] Area of Parasitology, Department of Biomedicine and Biotechnology, Universidad de Alcalá, Alcalá de Henares, 28805 Madrid, Spain.

[2] Road Ecology Lab, Department of Biodiversity, Ecology and Evolution, Faculty of Biology, Universidad Complutense de Madrid, 28040 Madrid, Spain.

[3] Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, and BIOPOLIS Program in Genomics, Biodiversity and Land Planning, Campus de Vairão, 4485-661 Vairão, Portugal.

[*] Corresponding author. E-mail address: sara.blazquezc@uah.es

cells (e.g., genera *Plasmodium*, *Hemolivia*, *Babesia*, and *Theileria*; Mehlhorn and Schein, 1985; Široký et al., 2007).

Although coinfections by heterogeneric parasites in single host organisms are common (e.g., Álvarez-Ruiz et al., 2018; Megía-Palma et al., 2018, 2022, 2023a; Zechmeisterová et al., 2019), to our knowledge double infections by heterogeneric blood parasites in single host cells have not been documented. A hybrid term to describe such an uncommon situation could be "coinfection (implying heterogenetic parasites) of a single host cell".

As part of an ongoing study of the phenology of blood parasites in the lizard *Psammodromus algirus* collected on the outskirts of Madrid, Spain (40.5167°N, 3.7833°W; elevation 650 m), we microscopically screened 12,000 blood cells per blood smear made from a total of 156 lizards, a sample of 1,872,000 screened cells in 2022. We found a total of 1524 infected cells, of which 837 were infected by gametocytes and trophozoites of the protozoan genus *Karyolysus* (Apicomplexa: Coccidia: Adeleorina) and 690 by sporozoites of the protozoan genus *Schellackia* (Apicomplexa: Coccidia: Eimeriorina). These two eukaryotic blood parasites have independent phylogenetic origins and can be morphologically differentiated (Megía-Palma et al., 2018; 2023a,b). *Schellackia* has one faintly bluish refractile body and *Karyolysus* has a characteristic banana-like shape, with throphozoitic stages having multiple digestive vacuoles, and gamonts typically embedded in an obvious parasitophorous vacuole (Beyer and Sidorenko, 1984). Furthermore, gametocyte infections are often associated with distortion of the host cell nucleus (Megía-Palma et al., 2023b). Recent research in the same population has suggested a differential recovery ability of the lizards from infections by these two genera of blood parasites (Megía-Palma et al., 2023a).

This unusual observation was made in a blood smear of an adult male *P. algirus*. In this sample, we observed 12 cells infected by *Karyolysus* (two trophozoites, five microgametocytes, five macrogametocytes) and 20 by *Schellackia* (mean ± standard error of the number of infected cells: *Karyolysus* = 9.08 ± 0.78, n = 90; *Schellackia* = 11.17 ± 2.42, n = 60). Multiple infections by a single parasite genus were observed in 1.9% of the cells in this blood smear (two sporozoites of *Schellackia* in a single erythrocyte and two more in an azurophilic cell). In contrast, the case of a single host erythrocyte infected by one sporozoite of *Schellackia* and one gametocyte of *Karyolysus* (Fig. 1) was detected only once and had a frequency of 6.56×10^{-4} (observed heterogeneric single host cell coinfections/infected erythrocytes) (Jovani and Sol, 2005). Coinfections of *Karyolysus* and *Schellackia* in lizards are relatively common, although not in single host cells. For example, in the same study area, 26.9% of hosts *P. algirus* were coinfected (Megía-Palma et al., 2022).

The observation reported here is noteworthy because it represents a rare case of double heterogeneric (co) infection in a single host erythrocyte. The low frequency of these observations in the sample suggests that the co-occurrence of one *Schellackia* sporozoite and one *Karyolysus* gametocyte within a single host erythrocyte would not benefit either the parasites or the host. We conclude that it represents a stochastic event of single host cell coinfection.

Acknowledgments. Permission for this work (license 10/184672.9/22) was kindly provided by the Dirección General de Biodiversidad y Recursos Naturales, Consejería de Medio Ambiente, Vivienda y Agricultura, Comunidad de Madrid. Patrimonio Nacional provided specific authorization to work in Monte de El Pardo. RM-P holds an autonomic permit to sample blood from small reptiles and enjoys a postdoctoral contract (CEECIND/04084/2017) provided by the Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto and Fundação da Ciência e Tecnologia. SB-C holds a contract by CAM and NextGenerationEU Funds (27-UAH-INV).

References

Ahmed, F.E., Mohammed, A.H.H. (1978): Studies of growth and development of gametocytes in *Haemoproteus columbae* Kruse. Journal of Protozoology **25**: 174–177.

Álvarez-Ruiz, L., Megía-Palma, R., Reguera, S., Ruiz, S., Zamora-Camacho, F.J., Figuerola, J., Moreno-Rueda, G. (2018): Opposed elevational variation in prevalence and intensity of endoparasites and their vectors in a lizard. Current Zoology **64**: 197–204.

Aparicio, G., Cordero del Campillo, M. (1980): Nota sobre *Karyolysus* sp. (Coccidia, *Haemogregarinidae*) del lagarto ocelado (*Lacerta lepida*). Anales de la Facultad de Veterinaria de León **26**: 101–106.

Beyer, T.V., Sidorenko, N.V. (1984): *Karyolysus* sp. (Haemogregarinidae, Adeleida, Apicomplexa): host-parasite relationships of persisting stages. Journal of Protozoology **31**: 513–517.

Franzén, L., Wahlin, B., Wahlgren, M., Aslund, L., Perlmann, P., Wigzell, H., Pettersson, U. (1989): Enhancement or inhibition of *Plasmodium falciparum* erythrocyte reinvasion in vitro by antibodies to an asparagine rich protein. Molecular and Biochemical Parasitology **32**: 201–212.

Inselburg, J. (1983): Gametocyte formation by the progeny of single *Plasmodium falciparum* schizonts. Journal of Parasitology **69**: 584–591.

Jovani, R. (2002): Malaria transmission, sex ratio and erythrocyte with two gametocytes. Trends in Parasitology **18**: 537–539.

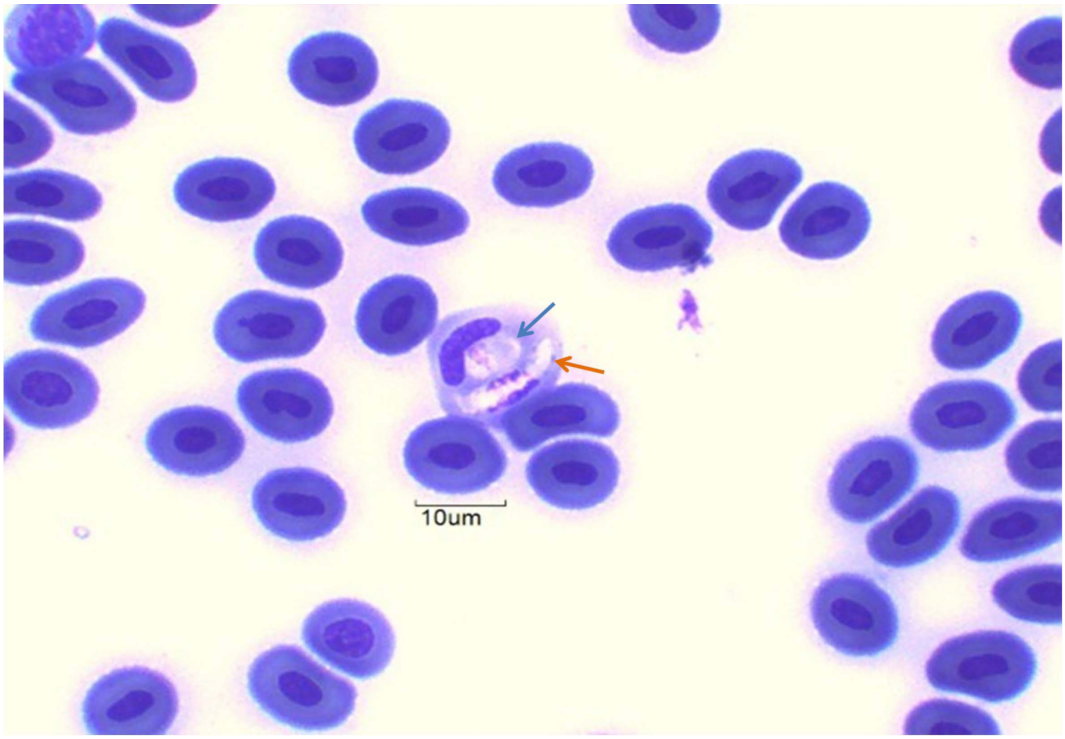

Figure 1. Microphotograph showing a single erythrocyte coinfected by *Schellackia* and *Karyolysus*. The blue arrow indicates the diagnostic refractile body in a *Schellackia* sporozoite next to the parasite nucleus; the latter structure is stained in faint purple. The orange arrow indicates a *Karyolysus* gametocyte.

Jovani, R., Sol, D. (2005): How predictable is the abundance of double gametocyte infections? Parasitology Research **97**: 84–86.

Jovani, R., Amo, L., Arriero, E., Krone, O., Marzal, A., Shurulinkov, P., et al. (2004): Double gametocyte infections in apicomplexan parasites of birds and reptiles. Parasitology Research **94**: 155–157.

Martínez-de la Puente, J., Merino, S. (2008): Mini-Review: factors affecting multiple invasions of erythrocytes in *Plasmodium* and other malaria-like parasites. A neglected characteristic of infections. The Open Parasitology Journal **2:** 40–42.

Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E. (2006): Are multiple gametocyte infections in malarial parasites an adaptation to ensure fertility? Parasitology **132**: 23–28.

Martínez-de la Puente, J., Merino, S., Tomás, G., Moreno, J., Morales, J., Lobato, E., García-Fraile, S. (2007): Can the host immune system promote multiple invasions of erythrocytes *in vivo*? Differential effects of medication and host sex in a wild malaria-like model. Parasitology **134**: 651–655.

Megía-Palma, R., Martínez, J., Merino, S. (2014): Molecular characterization of haemococcidia genus *Schellackia* (Apicomplexa) reveals the polyphyletic origin of the family Lankesterellidae. Zoologica Scripta **43**: 314–312.

Megía-Palma, R., Martínez, J., Cuervo, J.J., Belliure, J., Jiménez-Robles, O., Gomes, V., et al. (2018): Molecular evidence for host–parasite co-speciation between lizards and *Schellackia* parasites. International Journal for Parasitology **48**: 709–718.

Megía-Palma, R., Merino, S., Barrientos, R. (2022): Longitudinal effects of habitat quality, body condition, and parasites on colour patches of a multiornamented lizard. Behavioral Ecology and Sociobiology **76**: 73.

Megía-Palma, R., Redondo, L., Blázquez-Castro, S., Barrientos, R. (2023a): Differential recovery ability from infections by two blood parasite genera in males of a Mediterranean lacertid lizard after an experimental translocation. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology. In press.

Megía-Palma, R., Martínez, J., Fitze, P.S., Cuervo, J.J., Belliure, J., Jiménez-Robles, O., et al. (2023b): Genetic diversity, phylogenetic position, and co-phylogenetic relationships of *Karyolysus*, a common blood parasite of lizards in the western Mediterranean. International Journal for Parasitology **53**(4): 185–196.

Mehlhorn, H., Schein, E. (1985): The piroplasms: life cycle and sexual stages. Advances in Parasitology **23**: 37–103.

Miller, L.H., David, P.H., Hudson, D.E., Hadley, T.J., Richards, R.L., Aikawa, M. (1984): Monoclonal antibodies to a 140,000-m.W. protein on *Plasmodium knowlesi* merozoites inhibit their invasion of rhesus erythrocytes. Journal of Immunology **132**: 438–442.

Mutinga, M., Dipeolu, O. (1989): Saurian malaria in Kenya: description of new species of haemoproteid and haemogregarine parasites, anaplasma-like and pirhemocyton-like organisms in the blood of lizards in West Pokot District. International Journal of Tropical Insect Science **10**: 401–412.

Ramasamy, R., Yasawardena, S., Kanagaratnam, R., Buratti, E., Baralle, F.E., Ramasamy, M.S. (1999). Antibodies to a merozoite surface protein promote multiple invasion of red blood cells by malaria parasites. Parasite Immunology **21**: 397–407.

Roca, V., Galdón, M.A. (2010): Haemogregarine blood parasites in the lizards *Podarcis bocagei* (Seoane) and *P. carbonelli* (Pérez-Mellado) (Sauria: Lacertidae) from NW Portugal. Systematic Parasitology **75**: 75–79.

Shafeey, H.E.A., Mohamadain, H.S., Abdel-Gaber, R., Emara, N.M. (2019): Haemogregarines infecting reptiles in Egypt 1) Blood and merogenics stages of *Haemogregarine sp.* infecting the skink *Scincus scincus*. Egyptian Journal *of* Experimental Biology (Zoology) **15**: 127–133.

Široký, P., Kamler, M., Frye, F.L., Fictum, P., Modrý, D. (2007): Endogenous development of *Hemolivia mauritanica* (Apicomplexa: Adeleina: Haemogregarinidae) in the marginated tortoise *Testudo marginata* (Reptilia: Testudinidae): evidence from experimental infection. Folia Parasitologica **54**: 13–18.

Zechmeisterová, K., Goüy de Bellocq, J., Široký, P. (2019): Diversity of *Karyolysus* and *Schellackia* from the Iberian lizard *Lacerta schreiberi* with sequence data from engorged ticks. Parasitology **146**: 1690–1698.

Accepted by Saeed Hosseinian Yousefkhani