A new species of plasmodiidae (Coccidia: Hemosporidia) from the blood of the skink *Scincus hemprichii* (Scincidae: Reptilia) in Saudi Arabia

Mikky A. Amoudi, Mohamed S. Alyousif, Muheet A. Saifi, Abdullah D. Alanazi

**Abstract** *Fallisia arabica* n. sp. was described from peripheral blood smears of the Skink lizard, *Scincus hemprichii* from Jazan Province in the southwest of Saudi Arabia. Schizogony and gametogony take place within neutrophils in the peripheral blood of the host. Mature schizonts are rosette shaped 17.5 ± 4.1 x 17.0 ± 3.9 μm, with a L/W ratio of 1.03 (1.02–1.05) μm and produces 24 (18–26) merozoites. Young gametocytes are ellipsoidal, 5.5 ± 0.8 x 3.6 ± 0.5 μm, with a L/W of 1.53 (1.44–1.61) μm. Mature macrogametocytes are ellipsoidal, 9.7 ± 1.2 x 7.8 ± 1.0 μm, with a L/W of 1.24 (1.41–1.34) μm and microgametocytes are ellipsoidal, 7.0 ± 1.1 x 6.8 ± 0.9 μm, with a L/W of 1.03 (1.01–1.10) μm. In comparison to the described *Fallisia* species, this new taxon has rosette schizonts and is larger than *F. dominicensis*, in Hispaniola, *F. bipocrati*, *F. poecilopi*, in Panama, *F. thecadactyli* in Venezuela, and *F. effusa*, *F. simplex*, *F. modesta*, in Brazil. *F. arabica* has fewer merozoites than *F. effusa*, *F. poecilopi*, *F. thecadactyli* and *F. siamense* in Thailand. This new species has more merozoites than *F. dominicensis* and *F. modesta*. All of these species belong to diverse saurian families (Agamidae, Gekkonidae, Polychrotidae, Scincidae and Teiidae) parasitize only thrombocytes or lymphocytes and some species parasitize immature erythroid cells and leucocytes.

© 2014 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

1. Introduction

Limited information is available about hemosporidians of Saudi Arabia. The first attempts to shed some light on this matter were made by Al Sadoon and Bahrawy (1998), Al-Sadoon et al. (1999). Additional studies in this regard came recently from Al Farrag (2008) and Alyousif and Al-Shawa...
(2011). Fallisia was created by Lainson et al. (1974) but later considered as a synonym of Plasmodium (Levine, 1985). Telford (1986) then described Plasmodium siamense as a new species, which led to the resurrection of Fallisia as a subgenus of Plasmodium. Telford (1974, 1988) later recognized Fallisia as a genus of Plasmodiidae after Gabaldon et al. (1985) demonstrated the absence of an erythrocytic cycle following experimental transmission of an avian Fallisia sp. Three species of Fallisia have been described from Polychrotidae, i.e., F. biporcati, F. dominicensis, and F. poecilopi from the lizards Anolis biporcati, Anolis cybotes and Anolis poecilopus, respectively, and 1 species of Fallisia from a gekkonid, F. thecadactyli from the lizard Thecadactylus rapicaudus (Valkiunas, 2005). Fallisia simplex was described from an iguanid lizard Plica umbru and 2 species, F. effusa and F. modesta, from the lizards, Neusticurus bicarinatus and Tropidurus torquatus, respectively (Lainson et al., 1974, 1975). All of these species were described from Central and South America (Neotropical region). In addition to these species, F. copemani was described from a skink Carlia rhomboidalis in Australia and F. siamense from the flying dragon Draco maculatus in Thailand. Here, we describe a new species of Fallisia from the lizard, Scincus hemprichii, from Saudi Arabia.

2. Materials and methods

S. hemprichii is a small sand-colored scincid lizard, which lives in semi-arid desert localities, particularly in open areas, with few shrubs. Twenty-five S. hemprichii were collected during March 2011 from Jazan Province in Saudi Arabia which lies in the southwest corner of Saudi Arabia. Animals were identified according to Arnold (1986). Blood samples were taken by snipping the tail tip of the animals. Blood films were dried, fixed in methanol, and stained in 3% Giemsa stain prepared in phosphate buffer (PH 7.4) for at least 1 h then mounted in Canada balsam (Abdel-Ghaffar et al., 1994) and examined with a Zeiss-1 Universal Photo-Microscope III in 100× objective lenses. Measurements in μm were made by using a calibrated ocular micrometer.

3. Results

3.1. Taxonomic summary

Genus: Fallisia arabica n. sp. (Family, Garniidae)
Type host: S. hemprichii (Weigmann, 1837) (Squamata: Scincidae).

Site: Mature neutrophil cells of peripheral blood.
Type locality: Jazan Province, southwestern region of Saudi Arabia.
Prevalence: Eight of 25 (32%) lizards infected.
Etymology: The species name “arabica” was derived from the name of the country.
Type slide: Held in the Parasitological Collections of the Zoological Museum, College of Science, King Saud University, Riyadh, Saudi Arabia, as KSUC 555.

4. Description

This parasite infects principally mature neutrophil cells of peripheral blood. Double, triple, and numerous infections of single host cells are observed (Fig. 1). Host cell is distorted by the displacement of nucleus by schizonts and gametocytes. Unruptured mature schizonts are rosette shaped, often filling the entire host cell (Fig. 2), measuring 17.5 ± 4.1 × 17.0 ± 3.9 (n = 25), with a L/W ratio of 1.03 (1.02–1.05); 24(18–26) merozoites. Young gametocytes appear early in infection when schizogony ceases. Some of invading merozoites do not undergo nuclear division, but enlarge and become recognizable as young male and female gametocytes (Fig. 1). Young male and female gametocytes soon become distinguishable by staining reaction (Fig. 1). Gametocytes do not develop azurophilic granules in cytoplasm; they are ellipsoidal in shape and occupy the polar position of the host cell. Triple infections are seen, with 3 young gametocytes infecting the same host cell without distortion (Fig. 1). Young gametocytes 5.5 ± 0.8 × 3.6 ± 0.5 (n = 20), are with a L/W ratio of 1.5 (1.44–1.61) μm, mature microgametocytes 7.0 ± 1.10 × 6.8 ± 0.9 (n = 20), with a L/W ratio of 1.03 (1.01–1.10); delicate rose-pink in color, with scattered nuclear material and distinct nucleolus. Mature macrogametocytes 9.7 ± 1.2 × 7.8 ± 1.0 (n = 20); are with a L/W ratio of 1.24 (1.21–1.34); stained bright blue, sometimes with dense peripheral rim and a rather cyst-like appearance (Fig. 3). Nucleus is well defined. No azurophilic granules are seen in any of the gametocytes or asexual stages.

5. Discussion

There are 10 species of Fallisia, including this new species, F. arabica (Table 1). The description of the new taxon as a distinct species is based primarily on differences in structural features, host family, host specificity, and geographical distribution. F. arabica differs from all Fallisia species by...
| Species            | Host                  | Schizonts | No. of merozoites | Gametocytes | Microgametocytes | Macrogametocytes | Geographical distribution |
|--------------------|-----------------------|-----------|-------------------|-------------|------------------|------------------|--------------------------|
| F. biporcati       | Anolis biporcati      | Round or oval | 13.3 x 11.5 | 11 (9.1–38) | Triangular or Rectangular | 12.6 x 9.0 | 12.6 x 9.0 | Panama |
| F. copemani        | Carla rhomboidalis    | No data   | No data           | No data     | No data          | No data          | No data                  | Australia |
| F. dominicensis    | Anolis cybotes        | Round or oval | 6.0 x 4.8 | 12.4 (8–22) | Round, Oval or | 6.6 x 5.0 | 6.6 x 5.0 | Hispaniola |
| F. effusa          | Neusticurus bicarinatus | Round or Oval | 10.0 x 14.0 | 100 (50–150) | Elongate Oval | 8.0 x 5.0 | 10.0 x 6.0 | Northern Brazil |
| F. modesta         | Tropidurus torquatus  | Round or Oval | 5.5 x 5.0 | 16 (12–24) | Round or Oval | 7.0 x 5.5 | 7.5 x 5.5 | Northern Brazil |
| F. siamense        | Anolis poecilopus     | Oval or Elongate | 7.7 x 4.7 | 31.0 (20–51) | Round or Elongate | 10.1 x 8.0 | 10.1 x 8.0 | Panama |
| F. poecilopi       | Anolis poecilopus     | Oval      | 7.8 x 6.5 | No data       | Round           | 7.9 x 5.6 | 8.2 x 6.5 | Northern Brazil |
| F. simplex         | No data               | No data   | No data           | No data     | No data          | No data          | No data                  | No data |
| F. thecadactyl     | Thecadactylus rapicaudus | Oval, oblong or Triangular | 10.3 x 8.0 | 40.2 (26–61) | Round, Oval or Triangular | 10.4 x 7.0 | 10.4 x 7.0 | Panama and Venezuela |
| F. arabaica (This study) | Scincus hemprichii | Rosette     | 17.5 x 17.0 | 24 (18–26) | Ellipsoidal     | 7.0 x 6.8      | 9.7 x 7.8      | Saudi Arabia |
occurs primarily in neutrophils rather than thrombocytes or lymphocytes. The only species that infect neutrophils are Plasmodium audaciae (formerly Fallisia audaciae) (Edilene et al., 2006; Lainson et al., 1975) and Plasmodium leucocyctica, in Caribbean lizards. The new species differs from all Plasmodium species by the absence of hemozoin (Telford, 1974). P. arabica differs from P. audaciae by the absence of azurophilic granules in both asexual (schizonts) and sexual stages (gametocytes); the former species also produces far fewer merozoites (18–26) in comparison to 50–150. The new species differs from P. leucocyctica by the absence of azurophilic granules in both schizonts and gametocytes; schizonts are also smaller, i.e., 8–13 × 6–10 in P. leucocyctica in comparison to 17.5 ± 4.1 × 17.0 ± 3.9 in F. arabica. P. leucocyctica parasitizes at least 7 types of white blood cells including neutrophils, while F. arabica infects mainly neutrophils (schizonts and gametocytes); finally, F. arabica produces far fewer merozoites (18–26) in comparison to 43–80 in P. leucocyctica.

Genome analysis has created some confusion among taxonomists by challenging traditional systematic hypotheses, both within Plasmodium as well as relationships among the currently recognized genera (Escalante and Ayala, 1994; Escalante et al., 1995; Escalante et al., 1998; Martinsen et al., 2007; Perkins and Schall, 2002; Qari et al., 1996). One of the assertions suggests that a distinctive feature defining Plasmodium, i.e., schizogony in the vertebrate host erythrocytes, has been secondarily lost several times within the clade, signifying multiple shifting between avian and reptilian hosts (Perkins and Schall, 2002). While there is substantial genetic and morphological diversity among avian Plasmodium species (Bensch et al., 2004; Edilene et al., 2006 and Valkiunas, 2005), it seems that the number of reptilian Plasmodium species is still far behind compared to those of avian and mammalian hosts. However, a few of these lineages have been linked with morphospecies (Krizanauskiene et al., 2006; Sehgal et al., 2006). Some of the species in GenBank are misidentified not only at the species level, but at the generic and family levels as well (Valkiunas et al., 2008). We have concluded that the absence of pigment and azurophilic granules and the development of schizogony and gametogony in leucocytes by all species of Fallisia no doubt confirm that F. arabica possesses sufficiently unique features to distinguish it from other hemoplasmodian species.

**Competing Interests**

Authors declare no competing Interests.

**Acknowledgement**

This study was supported by King Saud University, the Deanship of Scientific Research, College of Science, Research Center.

**References**

Abdel-Ghaffar, A.F., El-Toukhy, A.A., Gawad, M., 1994. Light and electron microscope study on blood stages and merogony of haemogregarina species infected the gecko Ptyodactylus haselequistii. J. Egypt. Ger. Soc. Zool. 14, 341–363.

AL-Farrag, S., 2008. Light and electron microscopic study on a haemogregarine species infecting the viper Cerastes cerastes gasperitti from Saudi Arabia. Pak. J. Biol. Sci. 11 (11), 1414–1421.

Al-Sadoon, M.K., Alyousif, M.S., AL-Shawa, Y.R., Abdel-Ghaffar, F.A., 1999. Prevalence of haemogregariniae (Coccidia, Apicomplexa) infecting some snakes in Saudi Arabia and the host parasite relationship. J. Egypt. Ger. Soc. Zool. 28(D), 59–69.

Al-Sadoon, M.K., EL-Bahrawy, A.F., 1998. Blood parasites of five species of lizards trapped in Abha Province Saudi Arabia. J. Egypt. Soc. Parasitol. 28 (3), 899–905.

Alyousif, M.S., AL-Shawa, Y.R., 2011. Haemogregariniae (Coccidia: Apicomplexa) naturally infecting the skink Scincus hemprichii (Scincaeidae: Reptilia) from Saudi Arabia a light microscopic study. J. Egypt. Ger. Soc. Zool. 62(D), 83–94.

Arnold, E.N., 1986. A key and annotated checklist to the lizards and amphibians of Arabia. Fauna Saudi Arabia 8, 352–359.

Bensch, S., Perez-Tris, J., Waldenstrom, J., Hellgren, D., 2004. Linkage between nuclear and mitochondrial DNA sequences in avian malaria parasites: multiple cases of cryptic speciation. Evolution 58, 1617–1621. 

Edilene, O.J., Diniz, A.P., Lainson, R., Da Matta, R.A., de souza, W., 2006. Ultrastructural study of the gametocytes and merogonic stages of Fallisia audaciae (Haemosporina: Gamiidae) that infect neutrophils of the Lizard Plica umbra (Reptilia: Iguanidae). Protista 157, 13–19.

Escalante, A.A., Ayala, F.J., 1994. Phylogeny of the malarial genus Plasmodium derived from rRNA gene sequences. Proc. Nat. Acad. Sci. USA 91, 11373–11377.

Escalante, A.A., Barrio, E., Ayala, F.J., 1995. Evolutionary origin of human and primate malaria evidence from circumsoprozote protein gene. Mole. Biol. Evol. 12, 616–626.

Escalante, A.A., Freeland, D.F., Collins, W.E., Lal, A.A., 1998. The evolution of primate malaria parasites based on the gene encoding cytochrome b from the linear mitochondrial genome. Proc. Nat. Acad. Sci. USA 95, 8124–8129.

Gabaldon, A., Uitora, G., Zarpa, N., 1985. Fallisia (plasmodioides) neotropicalis subgen nov. sp. nov. from Venezuela. Parasitology 90, 217–225.

Krizanauskiene, A., Hellgren, O., Kosarev, V., Sokolov, L., Bensch, S., Valkiunas, G., 2006. Variation in host specificity between species of avian haemosporidian parasites: evidence from parasite morphology and cytochrome b gene sequences. J. Parasitol. 92, 1319–1324.

Lainson, R., Landau, I., Shaw, J.J., 1974. Further parasites of the family Gamiidae (Coccidia:Haemosporiidea) in Brazilian lizards. Fallisia effusa gen.nov. sp. nov. and Fallisia modesta gen.nov. Parasitology 68, 117–125.

Lainson, R., Shaw, J.J., Landau, I., 1975. Some blood parasites of the Brazilian lizards Plica umbra and Uranoscodion superciliosus (Iguanidae). Parasitology 70, 119–141.

Levine, N.D., 1985. Veterinary Protozoology. Iowa State University Press, Ames.

Martinsen, E.S., Waite, J.L., Schall, J.J., 2007. Morphologically defined subgenera of Plasmodium from avian hosts: test of monophyly by phylogenetic analysis of two mitochondrial genes. Parasitology 134, 483–490.

Perkins, S.L., Schall, J.J., 2002. A molecular phylogeny of malarial parasites recovered from cytochrome gene sequences. J. Parasitol. 88, 972–978.

Qari, S.H., Shi, Y.P., Pieniazek, N.J., Collins, W.E., Lal, A.A., 1996. Phylogenetic relationship among the malarial parasites based on small subunit rRNA gene sequences: monophyletic nature of the human malaria parasite Plasmodium falciparum. Mol. Phylogenet. Evol. 6, 157–165.

Sehgal, R.N.M., Valkiunas, T.A., Iezhova, Smith T.B., 2006. Blood parasites of chickens in Uganda and Cameroon with molecular descriptions of Leucocytozoon schoutedeni and Trypanosoma gallinarum. J. Parasitol. 92, 1336–1343.

Telford, S.R., 1974. The malarial parasites of Anolis species (Sauria:Iguanidae) in Panama. Int. J. Parasitol. 4, 91–102.
Telford, S.R., 1986. *Fallisia* parasites (Haemosporidia: Plasmodiidae) from the flying lizard, *Draco maculates* (Agamidae) in Thailand. J. Parasitol. 72, 766–769.

Telford, S.R., 1988. A contribution to the systematics of the reptilian malaria parasites. Family Plasmodiidae (Apicomplexa: Haemospororina). Bull. Fla. State Museum, Biol. Sci. 34, 65–96.

Valkiunas, G., Atkinson, C.T., Bensch, S., Sehgal, R.N.M., Ricklefs, R.E., 2008. Parasites misidentifications in GenBank: how to minimize their number. Trends Parasitol. 24, 247–248.

Valkiunas, G., 2005. Avian Malaria Parasites and Other Haemosporidia. CRC Press, Boca Raton, Florida.