Malaria Parasites: Species, Life Cycle, and Morphology

4

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Abstract

Plasmodium parasites are parasitized in vertebrates and female *Anopheles* mosquitoes. Although there are many different species, there are four main species of *Plasmodium* parasites in humans, namely, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae*. Furthermore, there is a fifth, rarer, human-monkey species, known as *Plasmodium knowlesi*. This chapter describes the biology, life history, and morphological characteristics of human *Plasmodium* to provide a comprehensive understanding of *Plasmodium* in humans.

Keywords

Human *Plasmodium* · Biological characteristics · Life cycle · Morphology

4.1 Etiology and Classification of Malaria

Malaria is an ancient infectious disease that poses a serious threat to human health. There are written records relating to malaria epidemics in China as far back as 3000 years ago. It was not until 1880 in Algeria that Charles-Louis-Alphonse Laveran, a French army doctor, first discovered *Plasmodium* in the blood of patients with fever by microscopic examination. In 1897, Ronald Ross, a British army doctor serving in India, confirmed that the *Anopheles* mosquito was the vector of malaria and elucidated the life cycle of the *Anopheles* mosquito and the mode of transmission of its bite. It was then that the mystery of malaria was unraveled.

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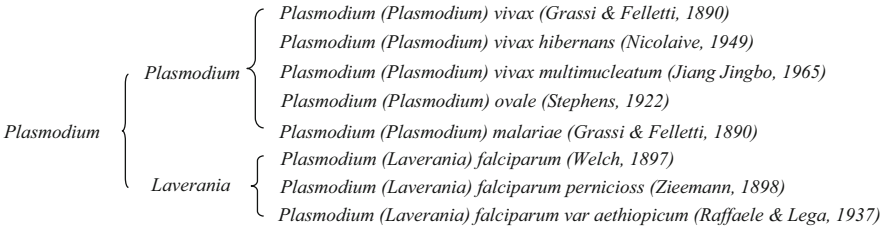

Fig. 4.1 The classification of main human *Plasmodium*

Plasmodium, the causative agent of malaria, is a unicellular eukaryotic protozoan belonging to Sporozoasida, the order Eucoccidiida, the family Plasmodidae, and the genus *Plasmodium*. There are many different species of *Plasmodium,* and the hosts can be amphibians, reptiles, birds, mammals, and other vertebrates, but the hosts of the species are highly specific, and there are significant biological differences between species. There are four main species of human malaria parasites, *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale*, *and Plasmodium malariae,* which cause falciparum malaria, Vivax malaria, Ovale malaria, and Quartan malaria, respectively. There are currently eight main named species (or subspecies), with *P. vivax, P. ovale*, and *P. malariae* belonging to the subgenus *Plasmodium* and *P. falciparum* belonging to the subgenus *Laverania* (Fig. 4.1). In addition, *P. ovale* contains two subtypes, *P. ovale curtisi* and *P. ovale wallikeri*, but it is controversial whether they are two subspecies. *P. falciparum, P. vivax,* and *P. ovale* are only parasitic in humans, whereas *P. malariae* can infect humans and apes in Africa (Xinping and Chuan, 2018; Shaowu et al., 2000; Sutherland et al., 2010).

Plasmodium knowlesi, a monkey *Plasmodium* endemic in the virgin forests of Malaysia, has been shown to cause natural infection from monkey to human, human to human, and human to monkey through mosquito vectors and is recognized as the fifth human *Plasmodium* species. However, *P. knowlesi* infections are less common, have a limited epidemiological range, and do not develop in humans as hyperparasitemia, hence the mild clinical symptoms of *P. knowlesi* infection in humans. Other rare cases of human infection with *Plasmodium simium, Plasmodium cynomolgi, Plasmodium schwetzi*, and *Plasmodium inui* have also been reported in the literature. The route and mode of infection and the degree of risk to humans have yet to be confirmed (Huaimin et al., 2006).

4.2 Life Cycle

The development and reproduction of *Plasmodium* require completion by vertebrates and insect vectors, and the hosts of human *Plasmodium* include humans and *Anopheles*. *Plasmodium* parasitizes human liver parenchyma cells and red blood cells (RBCs). Occasionally, *Plasmodium* may be present outside RBCs in peripheral blood smears, e.g., by released merozoites, or when hemolytic reactions occur

(Chap. 6, Fig. 6.14). In mosquitos, it parasitizes the mosquito stomach and finally gathers in salivary glands. The four major human *Plasmodium* species share a similar life cycle, which includes the exoerythrocytic (liver stage) and erythrocytic phases in humans and the gametogony and sporogony phases in *Anopheles* mosquitoes. Understanding the life cycle of *Plasmodium* is of great importance to the morphology of *Plasmodium* under the microscope (Xinping and Chuan, 2018; Xiaoqiu, 2007).

4.2.1 Development of *Plasmodium* in Humans

The exoerythrocytic phase is also known as the liver stage. When female *Anopheles* mosquitoes carrying *Plasmodium* sporozoites in salivary glands take a blood meal from humans, the sporozoites with saliva invade human peripheral blood, and they can remain under the skin for several hours. The majority of sporozoites then enter the capillaries, and a very small proportion invade the capillary lymphatics. The sporozoites follow the blood flow into the hepatic sinusoids, cross the Kupffer cell or sinusoidal endothelial cell space, and eventually invade the hepatic parenchymal cells. It takes approximately 30 minutes from the time the sporozoites enter the blood vessels to the time they invade the hepatocytes. Development and asexual schizogenesis of *Plasmodium* sporozoites are completed in parenchymal cells of the liver, and the development of *Plasmodium* to schizonts is termed schizonts of the exoerythrocytic phase or liver stage. The mature schizonts of the exoerythrocytic phase are 45–60 μm in diameter, which give birth to 10–30,000 merozoites, and escape from the hepatocytes in the form of merosomes, which bud out. After entering the peripheral blood, merosomes release merozoites, some of which are engulfed by macrophages and some of which successfully invade RBCs and begin the development of the erythrocytic phase. The duration of the exoerythrocytic phase varies in *Plasmodium* species, ranging from 6 to 12 days, with 5 to 6 days for *P. falciparum*, 8 days for *P. vivax*, 9 days for *P. ovale*, and 11 to 12 days for *P. malariae*. There are currently two genetically distinct types of sporozoites of *P. vivax* and *P. ovale*, namely, tachysporozoites and bradysporozoites. Tachysporozoites invade hepatocytes and continue to proliferate in the exoerythrocytic phase, releasing liver-stage merozoites that invade RBCs and cause clinical episodes through schizosomal proliferation. Bradysporozoites do not continue developing after invading hepatocytes temporarily but remain dormant (latent period). After a period of dormancy ranging from a few months to a few years (usually longer than 3 months), bradysporozoites develop into mature exoerythrocytic schizonts and release merozoites to invade RBCs to cause clinical symptoms, which is also known as "relapse." In Wuhan city, an overseas imported case infected with *P. ovale* that had a latent period of more than 14 months was observed, and a case with a latent period of more than 2 years was reported in China. Since no bradysporozoites are observed in either *P. falciparum* or *P. malariae*, there is no relapse of *P. falciparum* or *P. malariae*. However, in Wuhan city, several imported cases infected with *P. malariae* were found to present clinical symptoms after more

than 4 months of returning from overseas countries, which showed a similar latent period with *P. vivax* and *P. ovale*. In fact, the parasitemia density of *P. malariae* is generally lower than that of other species. The extremely low density causes long-term asymptomatic erythrocyte parasitism in *P. malariae*; however, clinical symptoms may occur due to the subsequently increased density.

The erythrocytic phase is also known as the blood stage or RBC stage. Following release to peripheral blood, some merozoites invade RBCs within a few seconds or minutes and develop in RBCs for reproduction by fission, while other merozoites are engulfed by phagocytic cells. The process of merozoite invasion into RBCs includes three consecutive stages. First, merozoites recognize and attach to receptors on the surface of the RBC membrane through specific sites. Second, RBCs are deformed, and the cell membrane is concave around merozoites to form *Plasmodium* vacuoles. Finally, the vacuoles are sealed after merozoite invasion. The merozoites develop into small trophozoites (ring trophozoites or trophozoites of the former forms) in RBCs. After swallowing hemoglobin and other nutrients, the nucleus is enlarged, the cytoplasm is increased, and iron ion-containing pigments are produced by breaking down hemoglobin. The color and shape of the malaria pigments vary somewhat between species of *Plasmodium*. Then, the small trophozoites develop gradually into large trophozoites (late trophozoites or mature trophozoites), also known as an amoeba-like shape, because of their amoeboid movements. The nucleus and cytoplasm of mature trophozoites begin to divide and develop into schizonts. Each nucleus of a mature schizont is surrounded by a piece of cytoplasm, giving it a grainy and clear state. The mature schizonts have 8–32 nuclei (merozoites), and the number of nuclei varies according to the species. When the schizonts are mature, the RBCs are exhausted and then broken. RBC fragments, merozoites, and malarial pigments are released into peripheral blood and cause the onset of clinical symptoms of malaria. Some merozoites are consumed by macrophages, while others reinvade new RBCs and begin a new erythrocytic phase. This cycle is known as the fission proliferation cycle. The duration of the erythrocytic phase varies with *Plasmodium* species, with 36–48 hours for *P. falciparum*, 48 hours for *P. vivax* and *P. ovale*, and 72 hours for *P. malariae*, producing a corresponding fever cycle. After more than ten hours of development in RBCs, the early trophozoites of *P. falciparum* gradually hide in microvessels, blood sinuses, and places with slow blood flow and continue to develop into late trophozoites and schizonts. Therefore, the late trophozoites and schizonts of *P. falciparum* are generally not easily found in peripheral blood, except for severe cases of falciparum malaria. After several fission proliferations of *Plasmodium* in RBCs, merozoites that have invaded RBCs no longer undergo asexual divisions but develop into female (macrogametocytes) and male gametocytes (microgametocytes). At this time, if female *Anopheles* bites and feeds on human blood, the mature female and male gametophytes are sucked into the mosquito's stomach, and sexual reproduction begins. If they remain in humans, they will age and die out spontaneously, being cleared out or simply engulfed by WBCs within 30–60 days. The immature gametophytes of *P. falciparum* are mainly in the microvessels and blood sinuses of the liver, spleen, bone marrow, and other organs. In general, they appear in the peripheral blood after maturity, and the time is

approximately 7–10 days after the emergence of asexual bodies in the peripheral blood. *P. falciparum* can parasitize all types of RBCs, *P. vivax* and *P. ovale* mainly parasitize reticulocytes, and *P. malariae* mostly parasitizes aged RBCs. In addition to new infections in malaria patients, the most common reason leading to the recurrence of clinical symptoms is the massive proliferation of residual *Plasmodium* in blood, which is called "resurgence." Therefore, no matter what kind of malaria parasite is infected, resurgence is occurring as long as the anti-malaria treatment fails.

4.2.2 Development of *Plasmodium* in Anopheles Mosquito

Gametogenesis: After *Plasmodium* at each stage enters mosquito stomachs by sucking the blood of malaria patients and *Plasmodium* carriers, mature female and male gametocytes continue to develop in the mosquito stomachs, while another asexual *Plasmodium* is digested, including small and large trophozoites, schizonts, and immature gametocytes. Female gametocytes form circular and inactive female gametes following nucleus meiosis. The nucleus of male gametocytes first divides into 4–8 pieces, and the cytoplasm extends 4–8 flagellate filaments, known as the filament phenomenon. Then, each nucleus enters a filament, which detaches from the mother and forms a flagellated male gamete. The male gamete may swim close to female gametes directionally. When in contact with the female gamete, it can burrow into the female gamete within seconds, forming a rounded zygote. The zygote develops into motile and elongated ookinetes. The ookinete passes through the epithelial cells on the mosquito gastric wall and stays between the epithelial cells and the outer elastic fibrous membrane, developing into an oocyst. If the patient's peripheral blood has a long interval between collection and production, then female and male gametes, the filament phenomenon (Figs. 6.25 and 6.57), ookinetes (Fig. 4.2), and other morphologies can also be observed in the peripheral blood smear.

P. vivax ookinetes may be present in human peripheral blood if the time interval from blood collection to the preparation of blood smears is long enough.

Sporozoite reproduction: The oocysts grow and protrude into the wall of the mosquito's stomach in the form of a tumor, and there can be several to dozens, or even more, of oocysts on the wall of the mosquito's stomach. The nuclei and cytoplasm in the oocysts are divided repeatedly and undergo sporozoite reproduction. Mature oocysts are approximately 40–60 μm in diameter and contain approximately 1000–10,000 spindle-shaped sporozoites, which are 10–15 μm long and approximately 1 μm wide, with a nucleus pointed at both ends and a curved body. The sporozoites can be released from the rupture or burrowed out of the oocyst and concentrated in the salivary glands of the mosquito via the hemolymph, developing into mature sporozoites. The duration of *Plasmodium* development in mosquitoes is related to temperature and humidity. The most suitable condition for *Plasmodium* sporozoites reproduction in mosquitoes is at a temperature of 24–26 °C and relative humidity of 75%–80%. A temperature lower than 16 °C or higher than 30 °C will

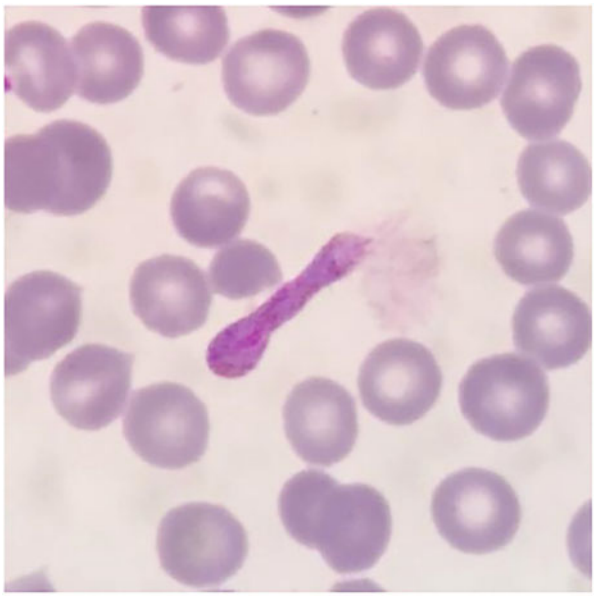

Fig. 4.2 A *P. vivax* ookinete (Giemsa staining, ×1000)

delay development and may cause degeneration until death. Mature sporozoites escape through the crevice of the oocysts or diffuse into the blood and eventually accumulate in the salivary gland adenocytes. If a female mosquito with sporozoites bites another person, sporozoites inject humans with saliva and initiate development in humans. Under the most suitable conditions, the development time of *Plasmodium* in *Anopheles* mosquitoes is 10–12 days for *P. falciparum*, 9–10 days for *P. vivax*, approximately 16 days for *P. ovale*, and 25–28 days for *P. malariae*.

The biological characteristics and life cycles of the four *Plasmodium* species are shown in Table 4.1 and Fig. 4.3.

4.3 Morphology of Human *Plasmodium* in the Erythrocytic Phase

The basic structure of *Plasmodium* includes the nucleus, cytoplasm and cell membrane. After ingestion of hemoglobin, malaria pigments, a product of digestion and breakdown of hemoglobin, appear in the pRBCs (Fig. 4.4). Blood smears with stainings, such as Giemsa or Wright's staining, show red or purplish red nuclei, blue or dark blue cytoplasm, and brown or black-brown malaria pigments. The four human *Plasmodium* species have the same basic structure, but the morphology of each stage of development varies. In addition to the morphological characteristics of the *Plasmodium* itself, the pRBCs can also change in morphology (Xinping and Chuan, 2018; Xiaoqiu, 2007).

Table 4.1 Biological characteristics of four human *Plasmodium* species

Plasmodium	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
Duration of the exoerythrocytic phase (Days)	5–6	8	9	11–12
Dormant body	No	Yes	Yes	No
Number of merozoites in the exoerythrocytic phase	30,000	10,000	15,000	15,000
Duration of schizogony in the erythrocytic phase (hours)	36–48	48	48	72
Characteristics of parasitized RBCs (pRBCs)	All types of RBCs	**Duffy Positive reticulocyte**[a] and normoblast	Reticulocyte and normoblast	Aged RBCs
pRBCs morphology	Normal, Maurer's clefts	Enlarged, Schüffner dots	Slightly enlarged, Schüffner dots	Normal or reduced, Ziemann dots
Malarial pigment	Black-brown	Brownish-yellow	Brownish-yellow	Dark-brown
Number of merozoites in the erythrocytic stage	8–26	12–24	6–12	6–12
Morphology of gametocytes	Crescent/ Sausage shape	Circular	Circular	Circular
Duration of development in mosquitoes at the most suitable conditions (Days)	10–12	9–10	16	25–28
Malarial pigments in oocyst	Chains and strips	Crown feather	Crossline	Gathered at the edge

[a] The merozoites released by *P. vivax* schizonts with Duffy binding protein (DBP) on their surface, which must bind to the Duffy antigen/receptor for chemokine (DARC) of reticulocytes before they can invade the RBCs. In contrast, Fy(a-b-) phenotype RBCs, which lack DARC, are protected from *P. vivax* infection or are at reduced risk of infection

Accumulation of malarial pigments is seen in leukocyte phagocytosis after medical treatment (red arrow), which is often observed in cases with high parasitemias or severe cases.

4.3.1 Morphological Characteristics of the Stages of *Plasmodium* Development in RBCs

The morphological characteristics of *Plasmodium* are grouped into three main developmental stages, namely, the trophozoite, schizont, and gametophyte stages, based on the morphological characteristics of each stage in the pRBCs. The

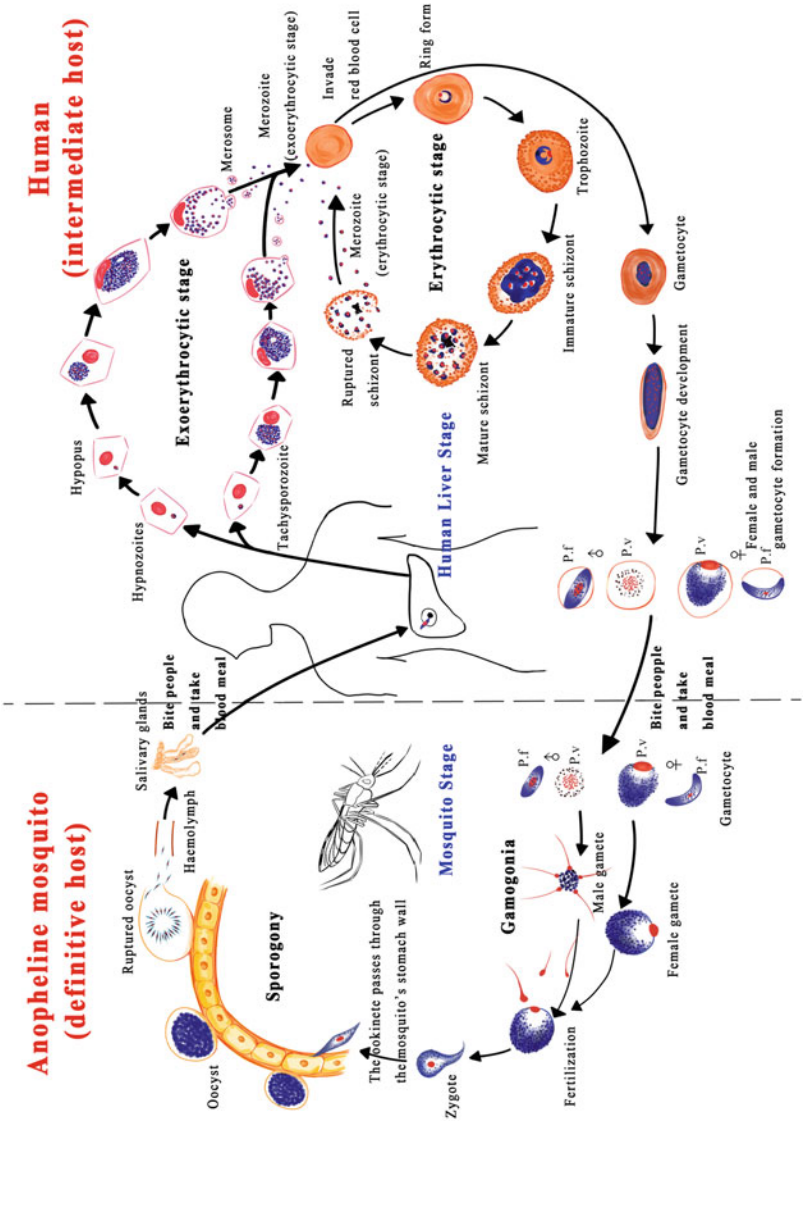

Fig. 4.3 The life cycle of *Plasmodium* species

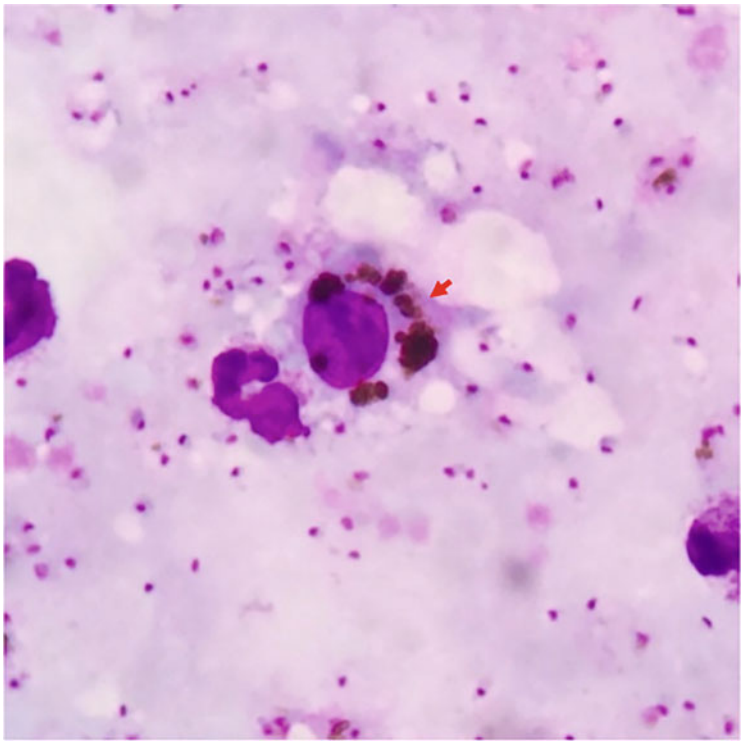

Fig. 4.4 Yellowish- or dark-brown malarial pigments (Giemsa staining, ×1000)

trophozoites and schizonts belong to the asexual stage, and the gametophytes belong to the sexual stage.

I. Trophozoites
Based on morphological characteristics, they can be subdivided into the small trophozoite stage and the large trophozoite stage. Small trophozoites, also known as ring-form and early trophozoites, have a small nucleus, little cytoplasm, a vacuole in the middle and a ring-shaped body. As *Plasmodium* feeds, grows, and develops, the nucleus and cytoplasm increase in size, and pseudopods and malaria pigments appear. Maurer's clefts appear inside pRBCs parasitized by *P. falciparum*, Schüffner's dots appear inside pRBCs parasitized by *P. vivax* and *P. ovale*, and Ziemann's dots appear inside pRBCs parasitized by *P. malariae.* At this point, *Plasmodium* develops into large trophozoites, which can also be called late trophozoites or mature trophozoites. The morphology and size of the large trophozoites vary considerably between *Plasmodium* parasites and are key to morphological differentiation.

II. Schizonts
As the trophozoites mature, the nucleus and cytoplasm begin to divide in a dichotomy called schizonts. The nucleus undergoes repeated divisions, and the

cytoplasm divides as well, with each nucleus surrounded by cytoplasm, called a merozoite. The schizonts can be subdivided into immature (early) schizonts and mature (late) schizonts. The nuclei of immature schizonts are few and tightly packed in undivided cytoplasm, whereas when there are more nuclei, the respective cytoplasm surrounds the nuclei and becomes grainy and clear, and the malaria pigment tends to concentrate; then, the schizonts are mature. The morphology of the schizonts and the number of schizonts vary considerably between *Plasmodium* parasites, which is the key to morphological differentiation.

III. Gametocytes

After *Plasmodium* parasites have undergone several fission proliferations and some merozoites invade RBCs, the nucleus and cytoplasm develop into round, oval or crescent-shaped, sausage-shaped gametophytes. Depending on morphological characteristics, gametophytes can be divided into female and male and can also be subdivided into immature gametophytes and mature gametophytes. The mature gametophyte of *P. falciparum* is crescentic and sausage shaped, which differs markedly from the round, oval-shaped gametophytes of *P. vivax, P. ovale*, *and P. malariae.*

4.3.2 Morphological Characteristics of Four Human *Plasmodium* Species in Thin Blood Smears

The morphological characteristics of *Plasmodium* in thin blood smears after Giemsa staining are shown in Table 4.2. The morphology of large (late or mature) trophozoites is sometimes similar to that of nearly mature female gametocytes. The identification is described in Table 4.3.

4.3.3 Morphological Characteristics of Four Human *Plasmodium* Species in Thick Blood Smears

The RBCs of the thick blood smears stacked cascade, *Plasmodium* shrank fold or part of *Plasmodium* was missing, the lysis of RBCs could not be used as a reference, and the hemolysis process also formed more impurities than thin blood smears, resulting in morphological identification being more difficult than thin blood smears. However, thick blood smears have more blood volume and a smaller area, and when RBCs are concentrated, the detection rate of *Plasmodium* is significantly higher than that of thin blood smears and less likely to be missed. The morphological characteristics of *Plasmodium* in thin blood smears after Giemsa staining are shown in Table 4.4.

Figures 4.5, 4.6, 4.7, and 4.8 describe the morphological characteristics of *Plasmodium* in thick and thin blood smears.

Table 4.2 Morphological identification of four *Plasmodium* species on a thin blood smear (Giemsa staining)

Plasmodium		*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
pRBCs	Size	Normal	Swelling	Normal or slight swelling	Normal or shrinking
	Shape	Normal	Polymorph	Oval or umbrella-arrow, comet-like the edge	Normal
	Color	Normal or slightly purple	Fading	Fading	Normal
	Dots	Uneven distribution of a few Maurer's clefts, which appear red and rough	Uniform distribution of lots of Schüffner's dots, which appear red and small	Uniform distribution of red Schüffner's dots, which are larger slightly than that of *P. vivax*	Light red Ziemann's dots
Former trophozoites (ring)	Size	Smaller, approximately 1/5–1/6 of the diameter of RBCs	Larger, accounting for approximately 1/3 of the diameter of RBCs	Smaller	Smaller
	Nucleus	1 or 2	1, 2 are rare	1	1
	Cytoplasm	Slender	Thin	Thicker	Thicker
	Malarial pigment	No	No	No	Occasionally tiny, brown particles
Large (late or mature) trophozoites	Size	Smaller	Larger	Medium	Medium
	Nucleus	1 or 2	1, 2 are rare	1	1
	Cytoplasm	Circular, nearly circular or ellipse, inapparent vacuoles	Amoeba-like, it often contains vacuoles	Circular, obvious vacuoles	Ribbon-like, inapparent vacuoles
	Malarial pigment	Small yellow-brown particles or formation of a black-brown block	Yellowish-brown, small, rhabditiform, scattered in distribution	Brownish and thick	Large dark-brown c distributed along the edges
Immature schizonts	Size	Smaller	Larger	Smaller	Smaller
	Nucleus	≥2	≥2	≥2	≥2
	Cytoplasm	Circular or nearly circular, disappearance of vacuoles	Circular or irregular, the disappearance of vacuoles	Circular or oval, the disappearance of vacuoles	Circular, the disappearance of vacuoles

(continued)

Table 4.2 (continued)

Plasmodium		*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
	Malarial pigment	Black-brown block	Yellowish-brown, uneven distribution or gathering in a heap	Brownish, uneven distribution	Dark-brown, uneven distribution
Mature schizonts	Size	Smaller than normal RBCs	Larger than normal RBCs	Less than normal RBCs	Less than normal RBCs
	Merozoites	8–32 but often 8–18, irregular arrangement, smaller	12–24 but often 16–18, irregular arrangement, larger	6–12 but often 8, irregular arrangement	6–12 but often 8, arranged in a flower-like manner
	Malarial pigment	Black-brown block	Yellowish-brown, often gathering in a heap on one side	Brownish, often gathering in a heap centrally or on one side	Dark-brown, often gathering in the center
Female gametocytes	Size	Larger	Larger than normal RBCs	Less than normal RBCs	Less than normal RBCs
	Shape	Crescent, sharp at both ends	Circular	Circular	Circular
	Nucleus	1, smaller, crimson, centered	1, larger, dense, crimson, located on one side	1, smaller, dense, crimson, located on one side	1, smaller, dense, crimson, located on one side
	Cytoplasm	Dark blue	Dark blue	Dark blue	Dark blue
	Malarial pigment	Black-brown, tight distribution around the nucleus	Yellowish-brown, evenly scattered	Brownish, scattered	Dark-brown, evenly scattered
Male gametocytes	Size	Larger	Larger than normal RBCs	Less than normal RBCs	Less than normal RBCs
	Shape	Sausage shape, blunt circle at both ends	Circular	Circular	Circular
	Nucleus	1, larger, light red, located in the center	1, larger, loose, light red, located in the center	1, larger, light red, located in the center	1, larger, light red, located in the center

	Cytoplasm	Light blue or light red	Light blue	Light blue	Light blue
	Malarial pigment	Black-brown distributed loosely around the nucleus	Yellowish-brown, evenly scattered	Brownish, scattered	Dark-brown, evenly scattered

Table 4.3 Morphological identification between large (late or mature) trophozoites and nearly mature female gametocytes

Plasmodium	Female gametocytes	Trophozoites
Size	Almost full of pRBCs	No more than 3/4 size of the pRBCs
Nucleus	One, large and dense, nonstained ribbons around	One, small or ribbon-like, no obvious nonstained ribbons around
Cytoplasm	The edges are clear without vacuoles	The edges are unclear and irregular, with vacuoles
Malarial pigment	More thick particles with even distribution, around nucleus	Less and tiny particles, uneven distribution

Table 4.4 Morphology of human malaria parasites on a thick blood smear

Plasmodium		*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
Trophozoites of the former (ring)		Small size. Consisted of slender cytoplasm and 1–2 smaller nuclei, always present "!", "spread the wings," "V" and "broken ring"	Larger size. Most have one larger nucleus, the cytoplasm is thicker, and always presents larger "!" or ","	The size is similar to that of the *P. vivax* with dense cytoplasm and a larger nucleus	Medium size. A larger nucleus and thick cytoplasm form "bird-eye" or "circle"
Large (late or mature) trophozoites	Size	Smaller	Largest among four species	The size is smaller than that of *P. vivax*	Medium size, it is slightly smaller than that of *P. ovale*
	Morphology	Often round or nearly circular	Amoeba-like, irregular shape, nucleus located in or outside the cytoplasm, which often shrinks into circles or breaks into pieces	The cytoplasm is dark blue, and the nucleus is larger	Often ribbon-like
	Malarial pigment	Tiny or gathered into 1–2 pieces	Tiny and yellowish-brown at an early stage, and then presenting rhabditiform or forming thick particles. The distribution is uneven	Pigment characteristics are similar to *P. vivax*	Thick particles and obvious
Schizonts		Smaller and 8–26 merozoites crowded together. Merozoites are also small	Larger and 12–24 dispersed merozoites. Merozoites are small or larger slightly	The size is smaller than that of *P. vivax* with 6–14 merozoites, larger and distributed closely	Smaller and 6–12 merozoites with scattered distribution. Merozoites are small
Gametocytes		Crescent shape of female gametocytes and sausage shape of male gametocytes, maybe folding or partial deletion	Largest among four species, circular and thick malarial pigments. Female gametocytes are larger, nucleus is also larger and leans to one side, cytoplasm is dark blue, male	The gametocyte morphology is similar to *P. vivax,* but the size is smaller than *P. vivax*	The gametocyte morphology is similar to *P. vivax* and *P. ovale,* but the size is more smaller. The malarial pigments are thicker

(continued)

Table 4.4 (continued)

Plasmodium	*P. falciparum*	*P. vivax*	*P. ovale*	*P. malariae*
		gametocytes are smaller than females, nucleus is enlarged and loose, and cytoplasm is light blue		
Malarial pigment	Tiny yellowish-brown particles at an early stage, then appearing as a black-brown block. The malarial pigment granules of gametophyte are thick and distributed around the nucleus	Tiny yellowish-brown at an early stage rod-like, or forming large, coarse particles. The distribution is uneven	The dark-brown large particles are dispersed	Sometimes small trophozoites with visible dark-brown pigments are thicker, which are distributed along the edges
RBCs	RBCs shadows and Maurer's clefts are seen	RBCs shadows and Schüffner's dots are often seen	Schüffner's dots are visible from the former trophozoites stage	RBCs shadows are seen
Others	Trophozoites of the former (ring) and gametocytes are often seen. Large trophozoites and schizonts are generally not seen	*Plasmodium* at all erythrocytic stages can often be found	*Plasmodium* is found at all erythrocytic stages	*Plasmodium* is found at all erythrocytic stages

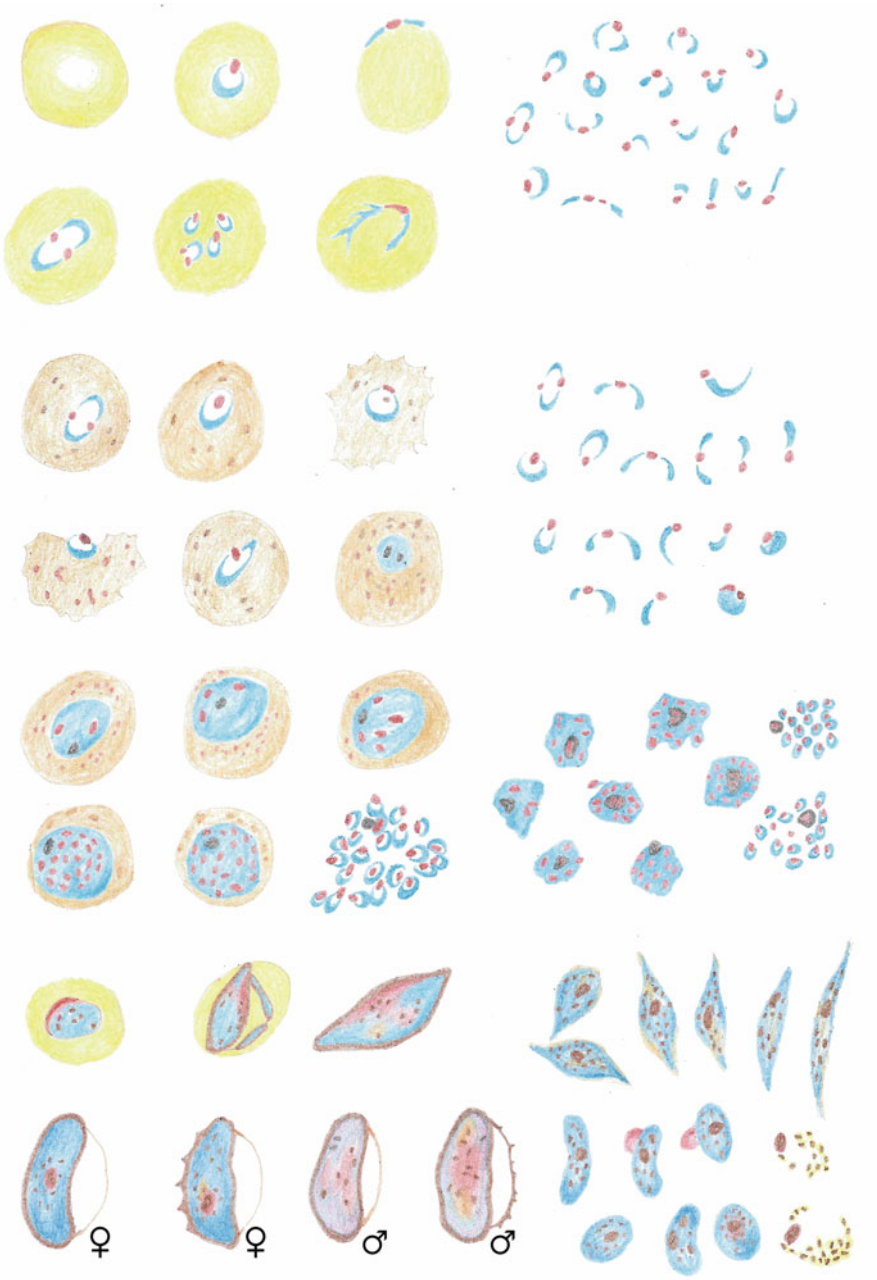

Fig. 4.5 Morphology of *P. falciparum* at the erythrocytic stage

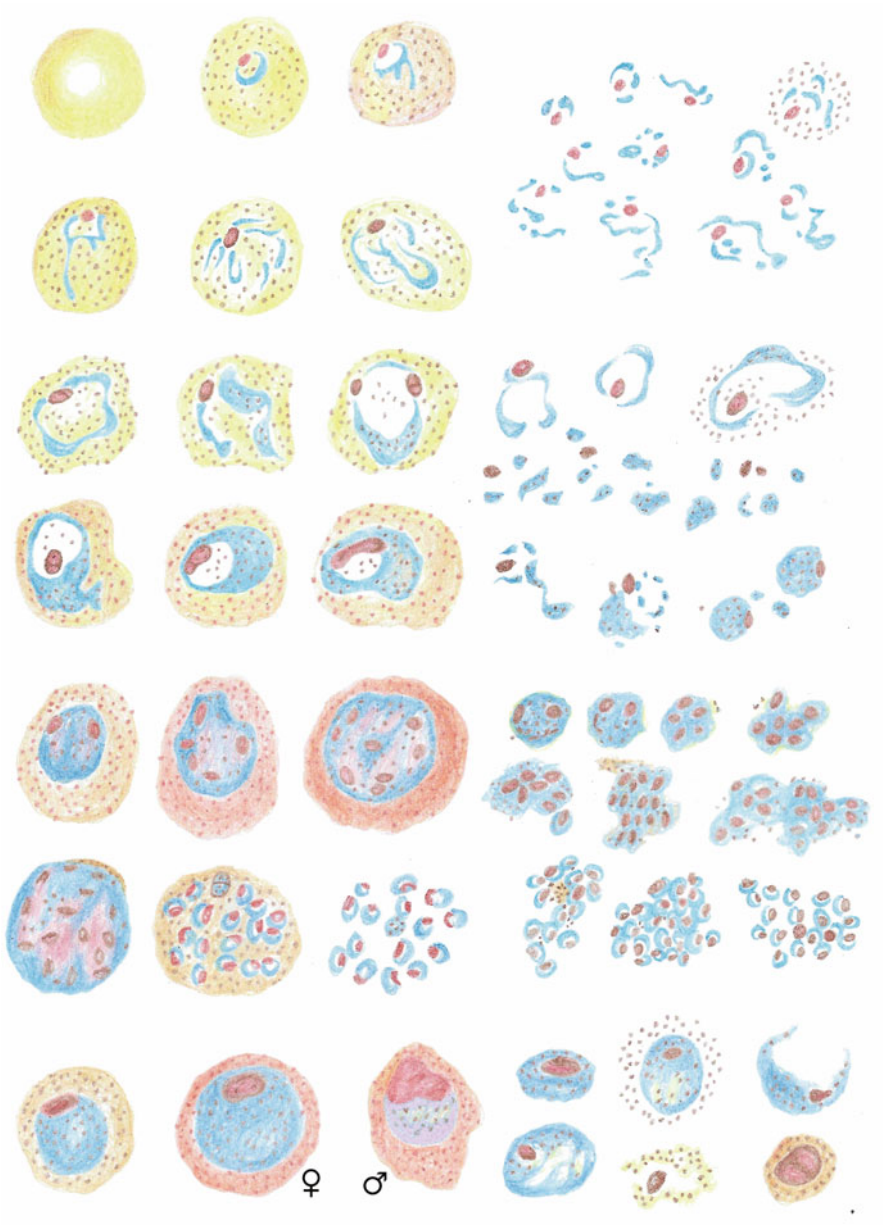

Fig. 4.6 Morphology of *P. vivax* at the erythrocytic stage

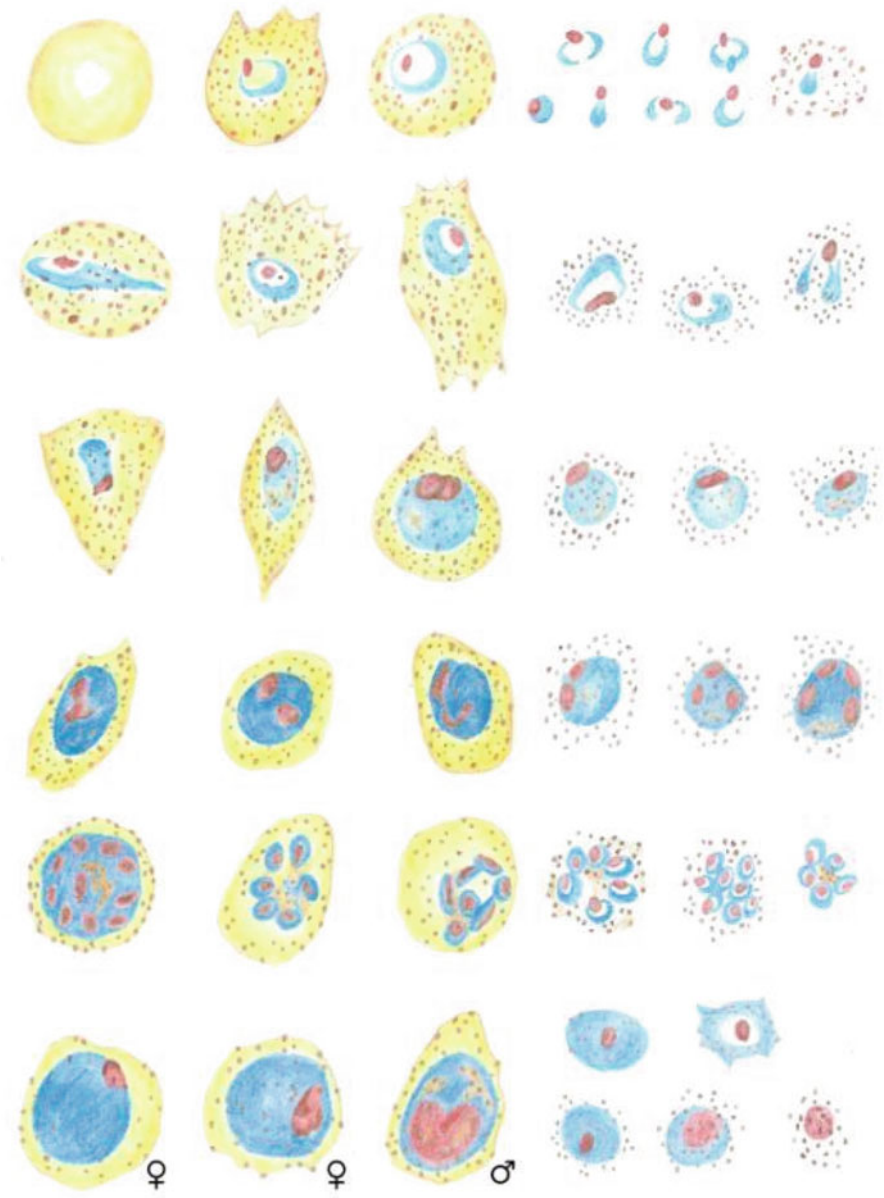

Fig. 4.7 Morphology of *P. ovale* at the erythrocytic stage

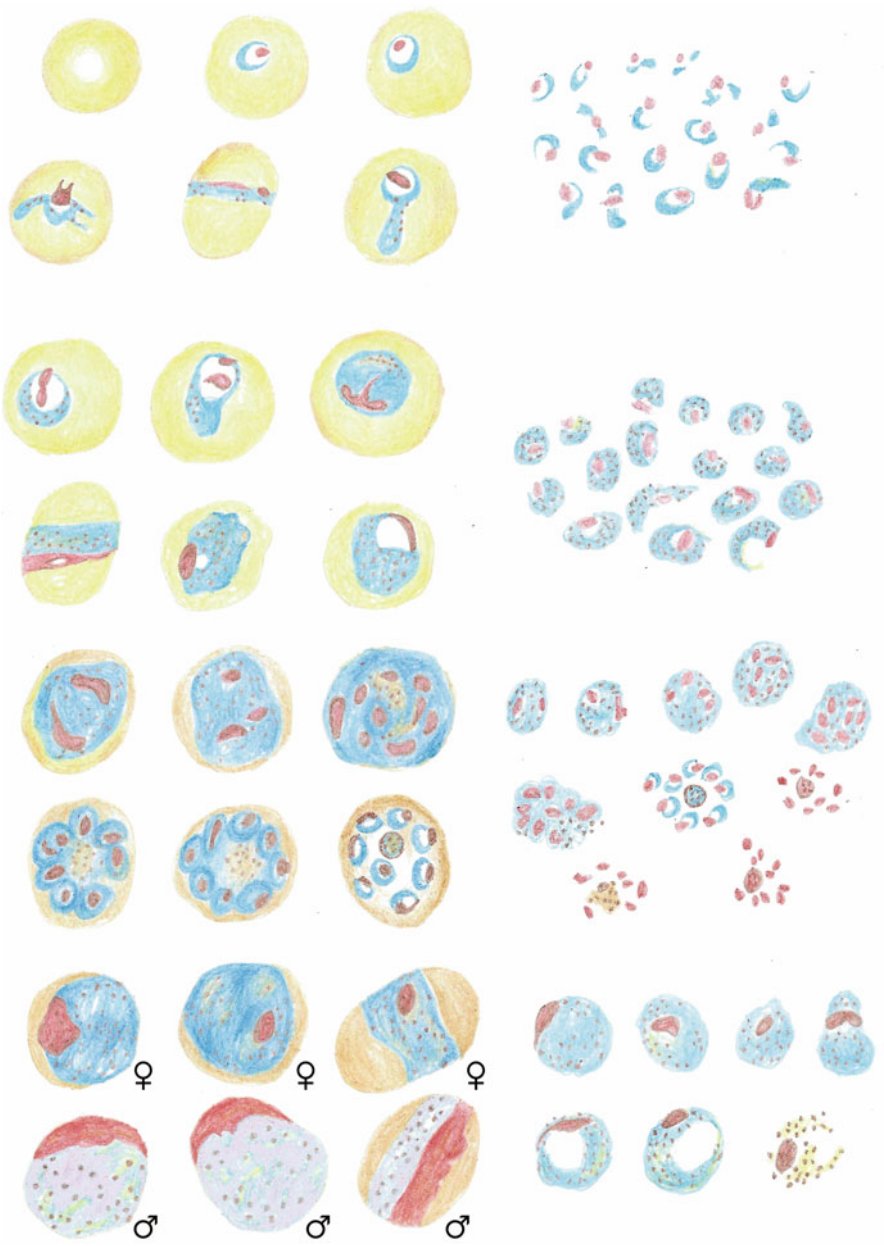

Fig. 4.8 Morphology of *P. malariae* at the erythrocytic stage

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