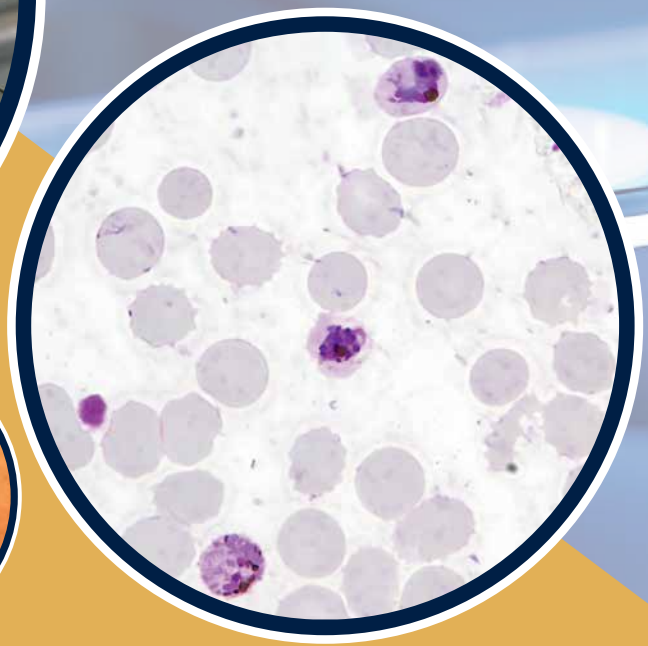
MINISTRY OF HEALTH MALAYSIA

Atlas of *Malaria Microscopy and Vectors* in Malaysia



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ATLAS
OF MALARIA MICROSCOPY AND VECTORS IN MALAYSIA

Published by:
NATIONAL PUBLIC HEALTH
LABORATORY

Cataloguing-in-Publication Data

Perpustakaan Negara Malaysia

A catalogue record for this book is available from the National Library of Malaysia

ISBN 978-967-13278-5-2

ATLAS OF MALARIA MICROSCOPY AND VECTORS IN MALAYSIA

Published by: National Public Health Laboratory, Ministry of Health, Malaysia.

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Suggested citation:
Atlas of Malaria Microscopy and Vectors in Malaysia (2025). National Public Health Laboratory, Ministry of Health, Malaysia.

The authors would like to thank the Director-General of Health, Malaysia for the permission to publish this document.

CONTENTS

ABBREVIATIONS

abd	:	abdomen
ASP	:	accessory sector pale
BFMP	:	blood film for malaria parasite
c	:	costa
CDC	:	U.S. Center for Disease Control and Prevention
Cu	:	cubitus vein, which is the fifth longitudinal vein
f	:	femur
Fe	:	femur
HD	:	humeral dark
MD	:	middle dark mark
mp	:	maxillary palp
NPHL	:	National Public Health Laboratory
p	:	proboscis
PCR	:	polymerase chain reaction
PHP	:	prehumeral pale spot
PSD	:	presector dark
PSP	:	presector pale spot
R	:	radius is the third longitudinal vein
RBC	:	red blood cell
SD	:	sector dark
SP	:	sector pale spot
sp.	:	species
t	:	tibia
ta	:	tarsus
w	:	wing
WHO	:	World Health Organization

The Ministry of Health in Malaysia has made significant strides in achieving the World Health Organization's Sustainable Developmental Goals and Universal Health Coverage, including the successful elimination of indigenous malaria cases since 2018. This accomplishment marks the end of the Malaria Elimination phase, and Malaysia has now entered the Prevention of Malaria Re-Establishment phase (POR).

Despite the global burden of malaria, with an estimated 241 million cases in 2020, Malaysia has reported only 229 imported human malaria cases in the same year. The country's success can be attributed to public-private healthcare partnerships that have improved quality, and lowered operational costs, demonstrating an efficient-driven healthcare model.

I would like to congratulate the WHO certified Level 1 competent malaria microscopists in the National Public Health Laboratory along with the Disease Control Division for documenting the microscopic morphology of the malaria parasites including the *P. knowlesi* and the entomology procedures for the usage of all health personnel. Finally, the Ministry of Health expresses its gratitude to all parties, especially Universiti Malaya and IMU University that have been involved in the development of the atlas.

Datuk Dr Muhammad Radzi bin Abu Hassan
Director-General of Health Malaysia

The Malaysian government, in collaboration with international partners and health organizations, recognizes the importance of maintaining vigilance against malaria even after achieving significant progress in its elimination.

Since 2018, Malaysia is committed to ensure the health and well-being of its citizens, against both human and zoonotic malaria. To effectively combat malaria, the Public Health fraternity needs to focus on strengthening malaria diagnostics, vector control measures and community health promotion and collaboration. This includes improving both microscopic and entomology tools with the development of this atlas.

In the face of climate change, which may contribute to the spread of vector- borne diseases, Malaysia is proactively preparing its healthcare infrastructure and policies. By addressing all forms of malaria and providing universal health coverage, the country aims to create a resilient healthcare system capable of dealing with emerging health threats.

In conclusion, the successful implementation of the atlas will prevent institutional memory loss in our quest to maintain the country's malaria- free status and to contribute to global efforts in combating this disease. The country's achievements serve as an inspiration for other nations working towards malaria elimination and emphasize the importance of collaboration, innovation and adaptability in public health.

Datuk Dr Norhayati Binti Rusli

Deputy Director-General of Health Malaysia (Public Health)

Assalamualaikum Warahmatullahi Wabarakatuh and Selamat Sejahtera

I am delighted to share with you the "Atlas for Malaria Microscopy and Vectors" sets the malaria parasitology and vector laboratorial work on all-malaria. This venture is between National Public Health Laboratory (NPHL), IMU University and Universiti Malaya to formally document best practices on Malarial Parasitology and Entomology in Malaysia.

This novel documentation will be a guide in malaria diagnosis and vector incrimination in our region. In contrast to human malaria that predominantly bites indoors, knowlesi malaria vectors are outdoor biters, making the importance to document observations and experience to benefit all junior health personnel.

I would like to thank the Malaria Reference Unit of the Parasitology Section and the Entomology Section, Disease Division of the NPHL for their unwavering perseverance and commitment to the overall malaria programme. Finally, I would like to extend my sincerest gratitude to Universiti Malaya, IMU University, the Vector Borne Disease and Entomology & Pest Sectors, Communicable Diseases Division of the Ministry of Health, Malaysia. With the dedication of our Health Ministry at all levels, combined with the support of our valued partners, I am confident that we will be able to effectively address the challenges posed by all forms of malaria in our country.

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INTRODUCTION

The Atlas of Malaria Microscopy and Vector Incrimination in Malaysia serves as a vital resource for understanding the diverse malaria parasites, particularly their morphological features and the vectors involved in their transmission. Despite achieving zero indigenous human malaria cases since 2018, the emergence of *Plasmodium knowlesi*, a zoonotic malaria parasite with long-tailed macaques *(Macaca fascicularis)* and pig-tailed macaques *(Macaca nemestrina)* as its natural hosts, underscores the ongoing relevance of malaria surveillance and education.

Malaria in Malaysia has largely been curtailed through effective control and management strategies aimed at human malaria parasites, namely *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale.* However, the rise in *P. knowlesi* infections poses new challenges, as this parasite is transmitted to humans via Anopheles mosquitoes, highlighting the intertwined relationships between human activities, wildlife habitats, and disease transmission dynamics.

The atlas plays an essential role in preserving the morphological characteristics of malaria parasites that were historically present in Malaysia and addresses the complexities in malaria microscopy, especially in diagnosing *P. knowlesi.* The preparation, Giemsa-staining and examination of the BFMPs for the diagnosis of malaria parasite species and density are in accordance to the Malaria Microscopy Standard Operating Procedures by the WHO (WHO, 2016). In Malaysia, molecular surveillance is further implemented to verify malaria parasites species, including *P. knowlesi.*

Vector incrimination can be assessed by examining mosquitoes collected from the field, followed by species identification and dissection. The presence of Plasmodium sporozoites in the salivary glands and oocysts in the midgut confirms their role in malaria transmission. However, the parity rate is assessed to determine mosquito longevity, reproductive status and vectorial capacity. If the

mosquitoes were found positive for Plasmodium parasites, they should be transferred into 90% alcohol so that polymerase chain reaction (PCR) can be conducted to determine the species of Plasmodium. This systematic approach ensures a comprehensive entomological assessment of Anopheles mosquitoes, providing valuable data to enhance malaria control efforts in the country. This book presents a simplified and modified identification key based on the taxonomic classifications of Reid (1968) and Sallum & Peyton (2005) together with mosquito morphological common feature, aiding entomologists in the identification of major malaria vector species in Malaysia.

By documenting the morphological similarities and differences between different malaria parasite species and providing a taxonomy key for the Leucosphyrus group of Anopheles mosquitoes, the atlas contributes significantly to the fields of parasitology and entomology. It offers an updated information to guide diagnosis and assist entomologists. In summary, existing microscopy diagnostic techniques, vector identification and dissection processes in this atlas should be used in line with the recommended WHO bench aids and related taxonomy procedures. Overall, the atlas stands as a pictorial educational tool aimed at bolstering malaria control efforts and enhancing our understanding of zoonotic infections in Malaysia.

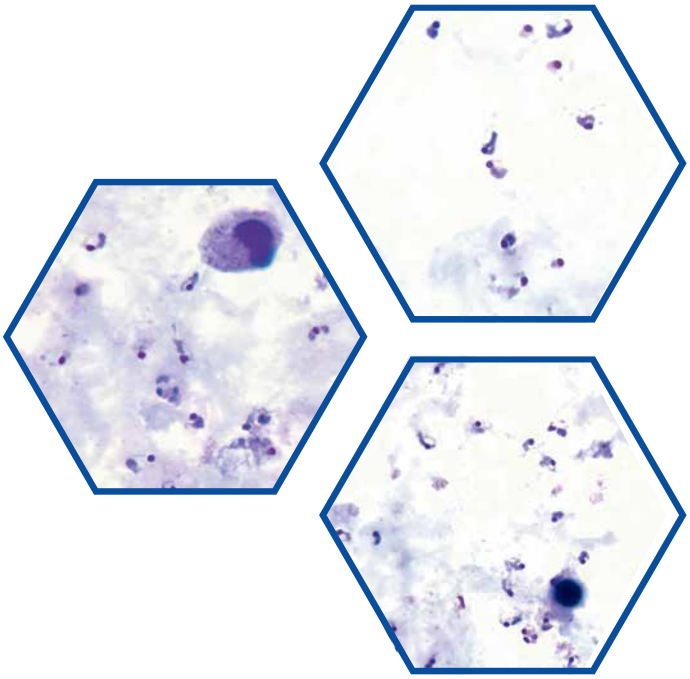
Chapter 1

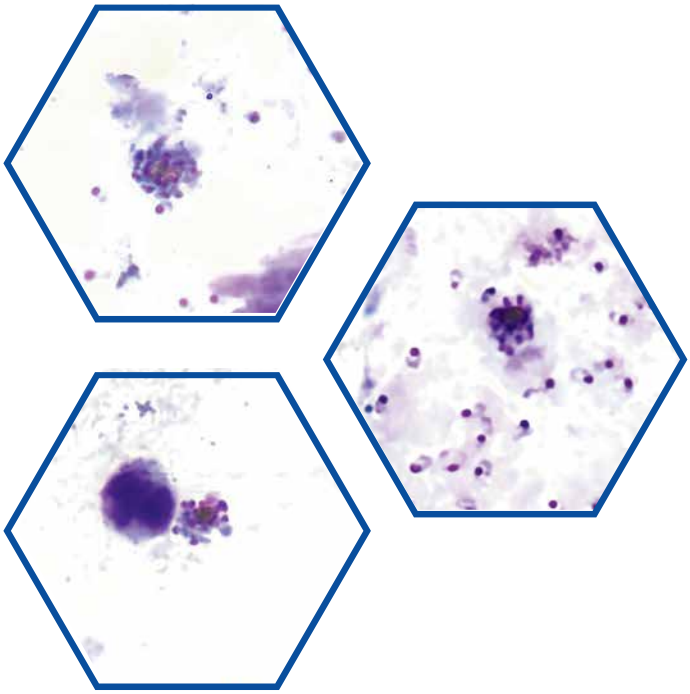
MALARIA MICROSCOPY

GIEMSA- STAINED THIN AND THICK BLOOD FILMS

THICK BLOOD FILM
Plasmodium falciparum

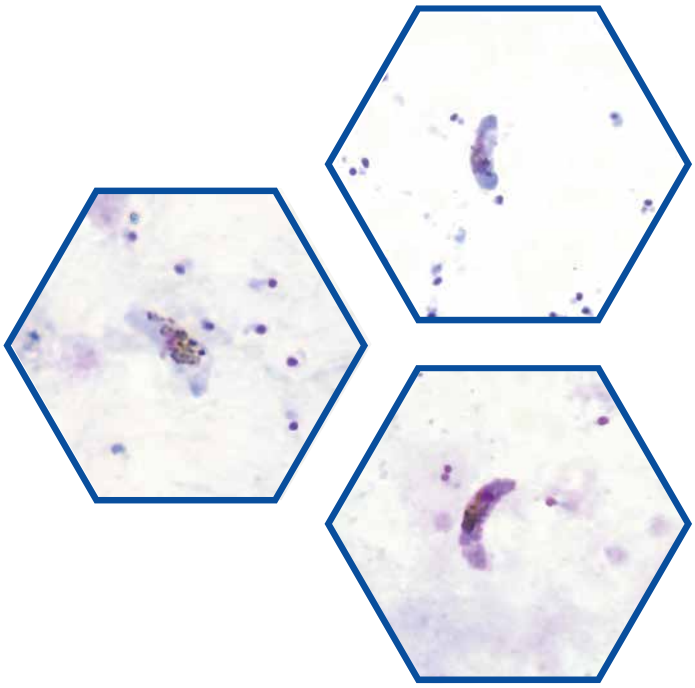
TROPHOZOITES
Trophozoites appear uniform with various shapes such as the ring-form, comma-form, i-form and headphone-form. Chromatin can appear single or double accompanied by fine cytoplasm.





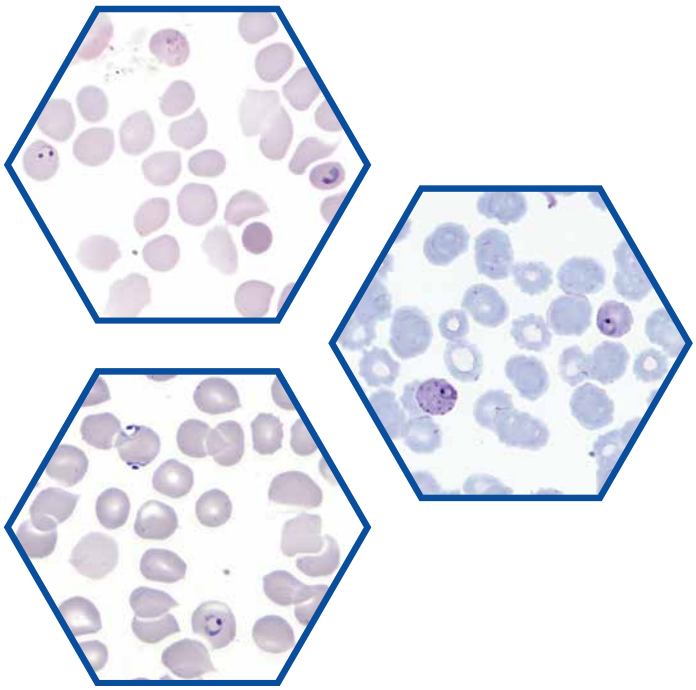
SCHIZONTS
Schizonts appear as a concentrated mass of pigment with 8-24 merozoites in a compact cluster. However, *P. falciparum* schizonts are rarely seen in BFMP and is often observed with high parasitemia.

GAMETOCYTES
Gametocytes of *P. falciparum* are easily distinguishable from other malaria parasite species due to their distinctive crescent and banana shapes. The pigment is observed as a coarse and scattered central mass.

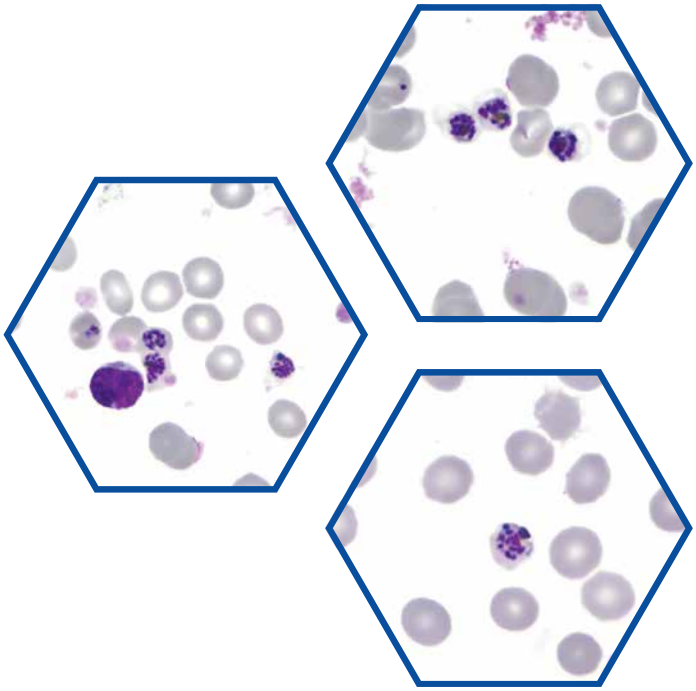


Reference:
WHO 2010 ; CDC Website

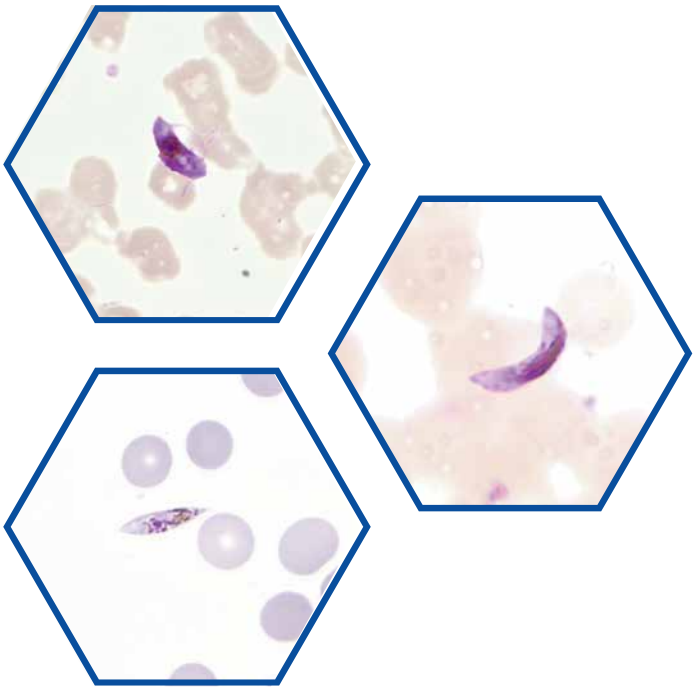
THIN BLOOD FILM
Plasmodium falciparum



TROPHOZOITES
Infected RBCs are not enlarged and remain normal in size. When the BFMP is well stained, Maurer's Cleft may be observed on the infected RBCs. Trophozoites of *P. falciparum* can sometimes be seen in the periphery of the infected RBCs. Multiple infections of a single RBC are also commonly observed.



SCHIZONTS
Infected RBCs are almost completely filled by schizonts. Merozoites are seen in a compact cluster with a mass of pigment.

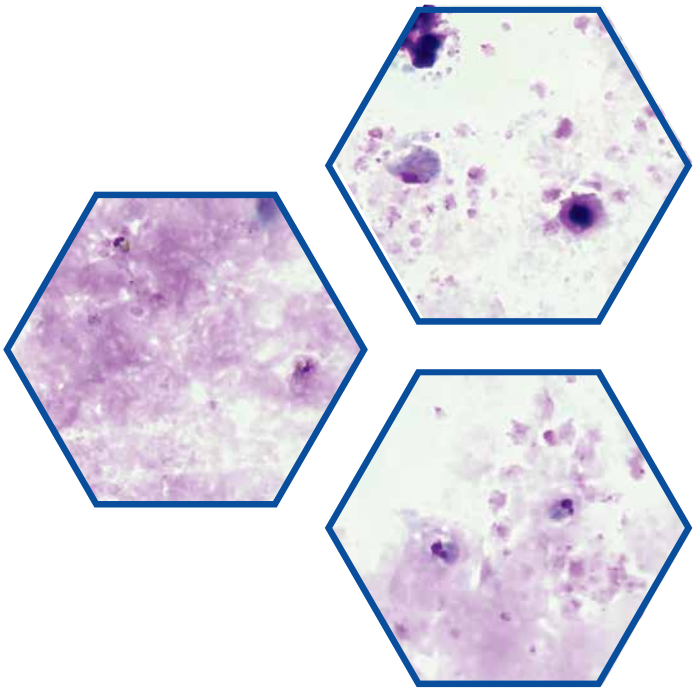


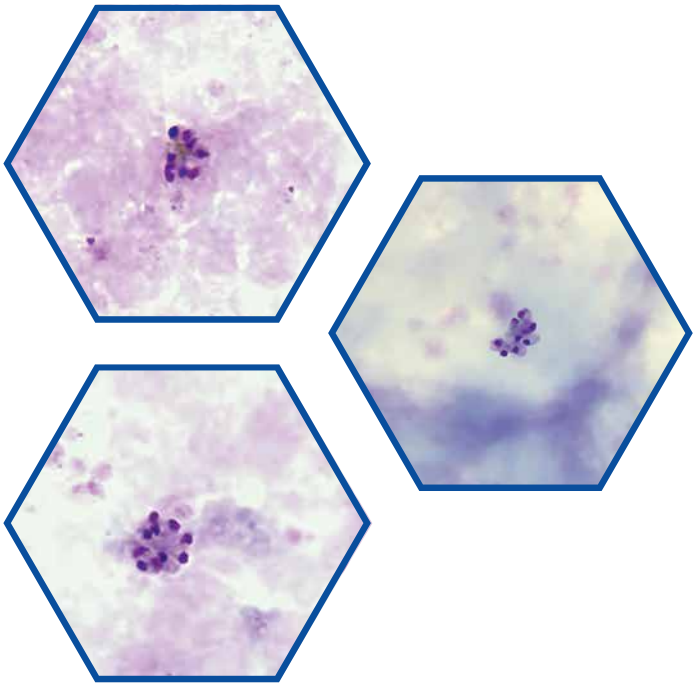
GAMETOCYTES
Gametocytes are crescent or banana shaped with a central coarse mass. The membranes of the RBC that has been infected with the gametocyte may not always be observed.

Reference:
WHO 2010 ; CDC Website

THICK BLOOD FILM
Plasmodium malariae

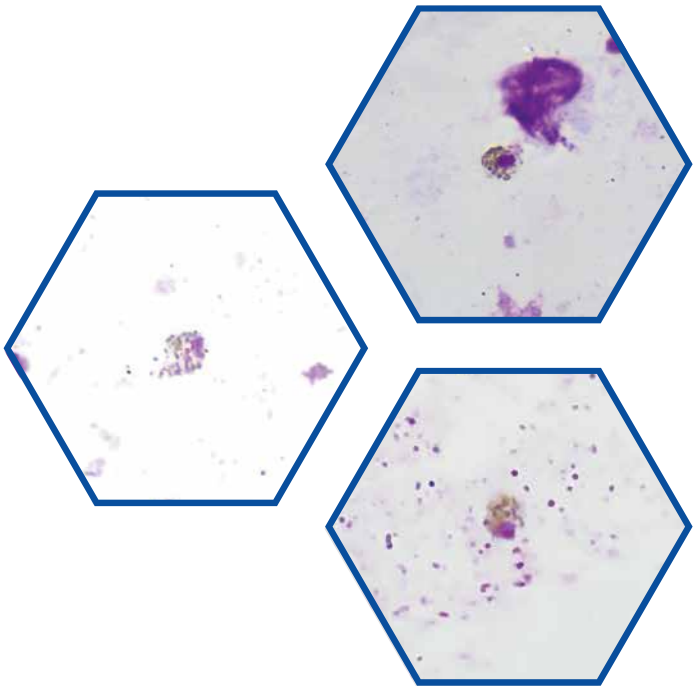
TROPHOZOITES
Trophozoites are small with compact cytoplasm and a single, prominent chromatin. Coarse dark brown granules with yellow tinge are usually seen in the cytoplasm.





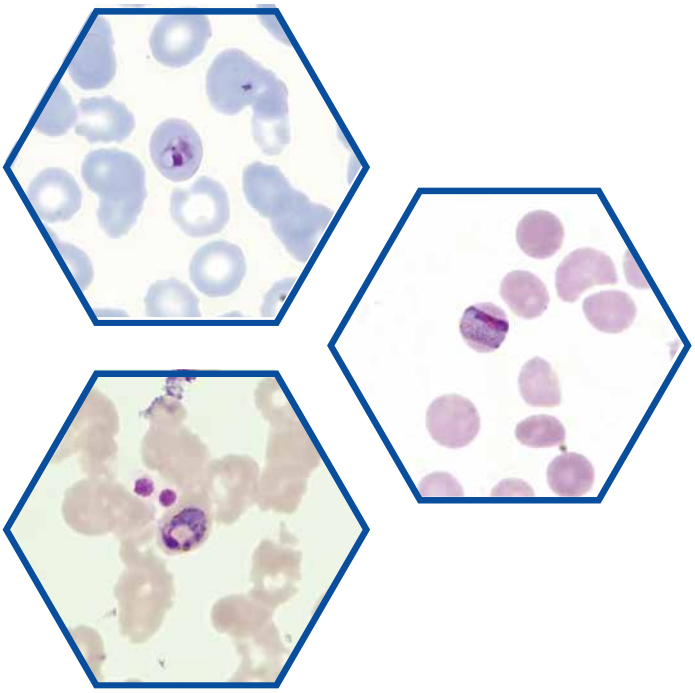
SCHIZONTS
The schizonts have 6-12 merozoites, distinctively arranged in a 'rosette' formation surrounding a yellow-tinged pigmented mass.

GAMETOCYTES
Gametocytes are distinguished from mature trophozoites by the presence of a single and enlarged chromatin. Dark brown pigments are scattered throughout the gametocytes.

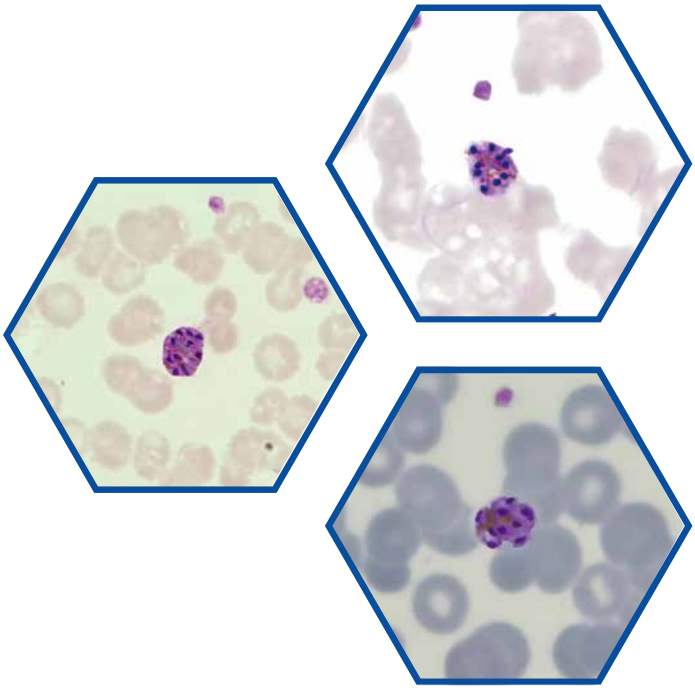


Reference:
WHO 2010 ; CDC Website

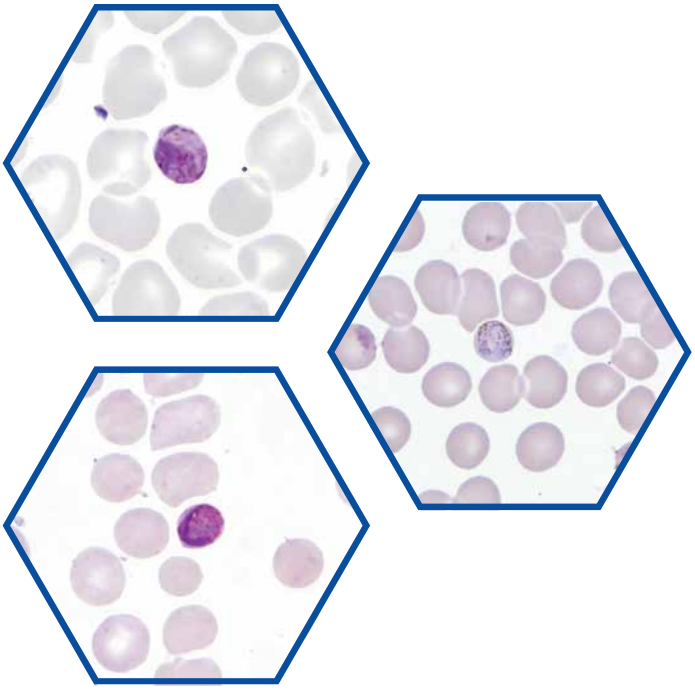
THIN BLOOD FILM
Plasmodium malariae



TROPHOZOITES
The infected RBCs are not enlarged but sometimes reduced in size. The trophozoites appear in many shapes such as the 'bird's-eye' form, basket-form and the band-form.



SCHIZONTS
Schizonts usually take up the entire RBC and consists of 6- 12 merozoites. The arrangement of merozoites can be seen in a 'rosette' formation surrounding a yellow-tinged pigmented mass.

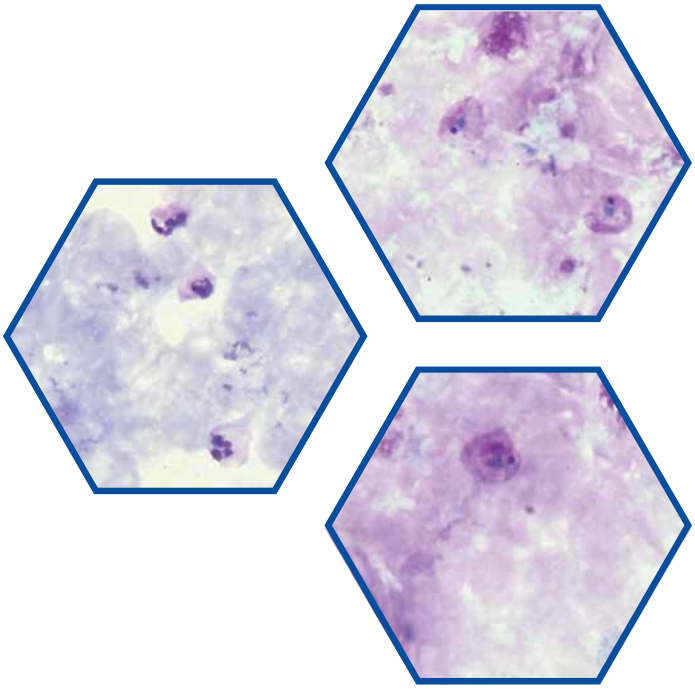


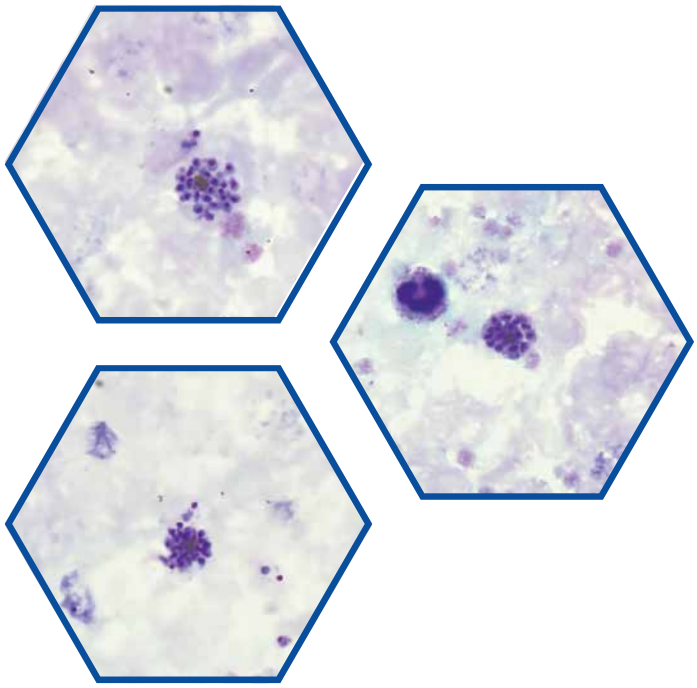
GAMETOCYTES
The gametocytes may fill the entire RBC. The chromatin is enlarged and usually located eccentrically. The dark brown pigment is dispersed throughout the cytoplasm.

Reference:
WHO 2010 ; CDC Website

THICK BLOOD FILM
Plasmodium vivax

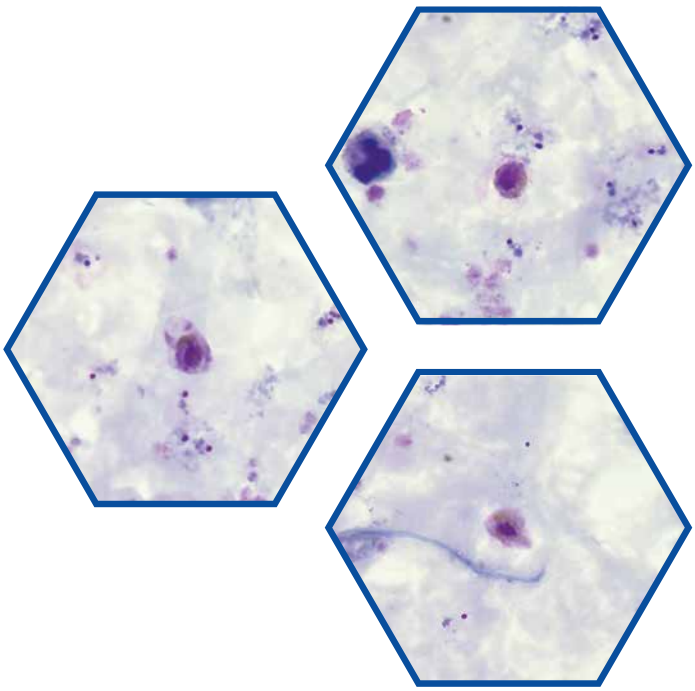
TROPHOZOITES
Trophozoites are contained within the ghost cells, which are characterized by their pinkish and translucent appearance. The cytoplasm of the trophozoites are irregular, fragmented or amoeboid in shape while the chromatins are large and prominent.





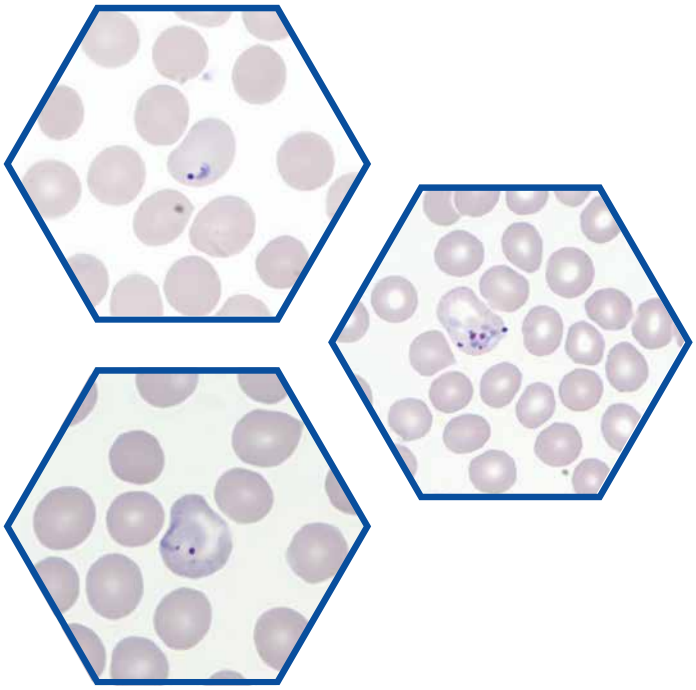
SCHIZONTS
Schizonts are in a cluster of 12-24 merozoites accompanied by a mass of pigment.

GAMETOCYTES
Gametocytes are larger compared to other human malaria parasite species, appearing round and compact. The chromatins are enlarged and located eccentrically. The cytoplasm of the parasite is uniform and scattered with pigments.

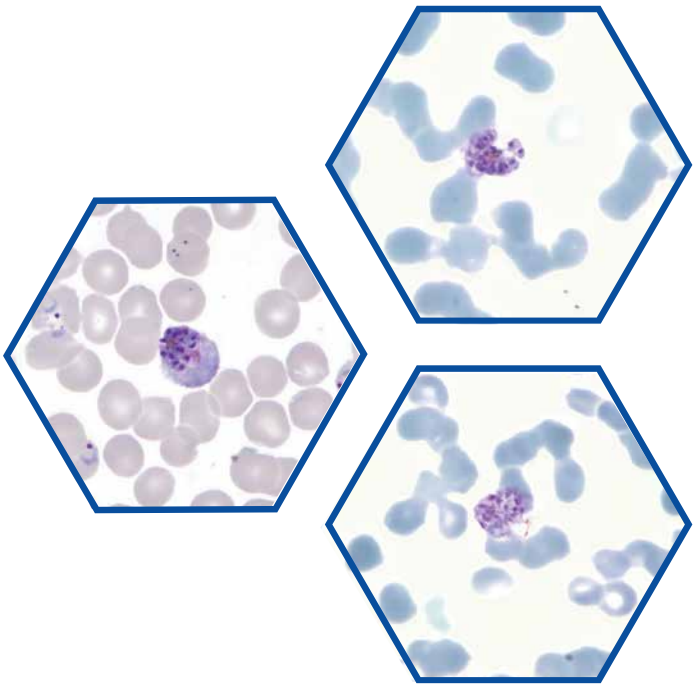


Reference:
WHO 2010 ; CDC Website

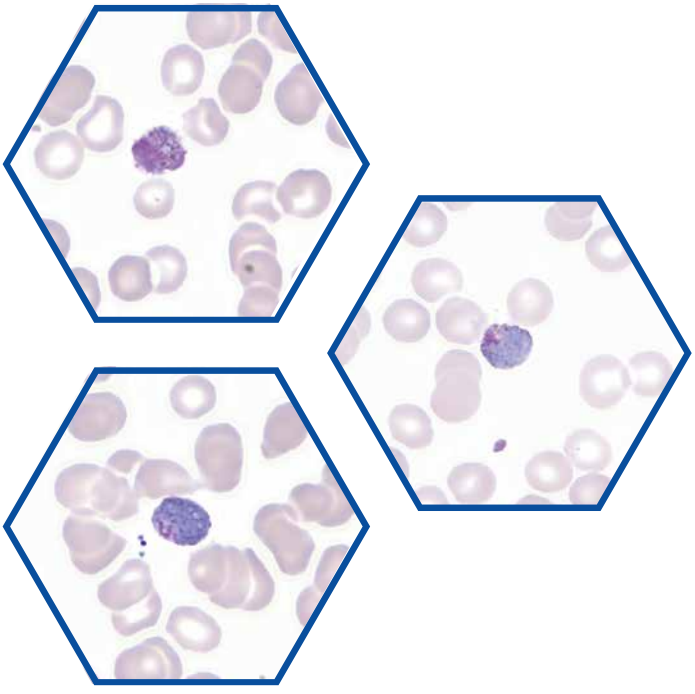
THIN BLOOD FILM
Plasmodium vivax



TROPHOZOITES
Infected RBCs are typically enlarged and distorted. Fine stippling may also be observed. The early trophozoites have delicate rings while the matured trophozoites may become more amoeboid in shape. The chromatins of the parasite are usually large and prominent.



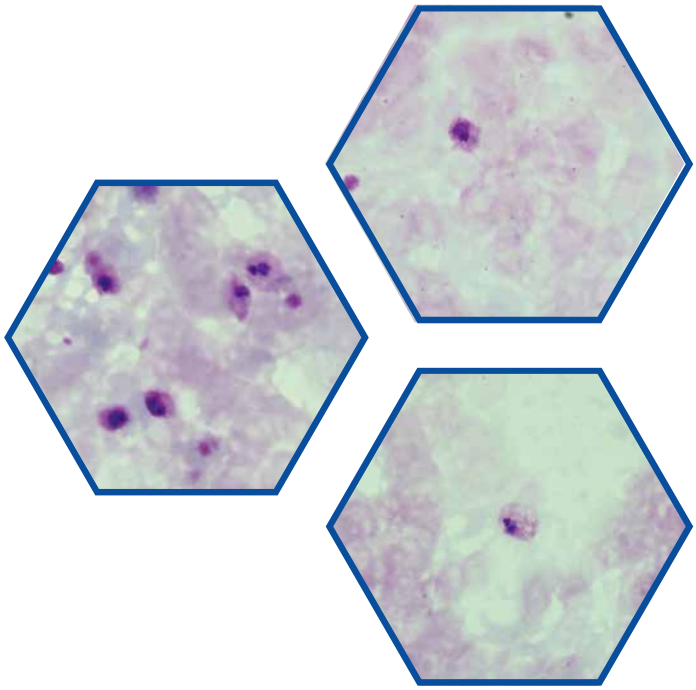
SCHIZONTS
Matured schizonts are known to have 12-24 merozoites in irregular clusters accompanied by loose pigmented mass. Infected RBCs are enlarged in size and are almost fully occupied by the schizonts.



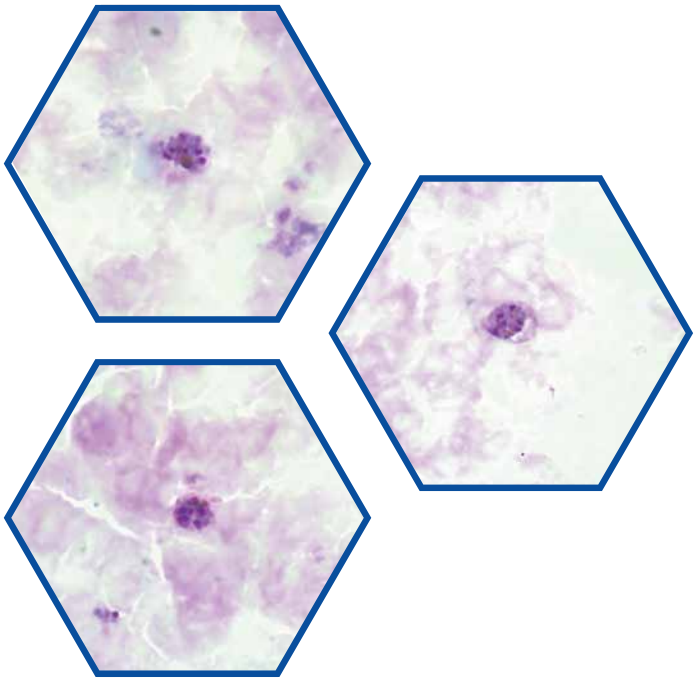
GAMETOCYTES
Gametocytes often fill the entire infected RBC. The chromatin is well defined and located eccentrically. The cytoplasm of the parasite is homogenous and scattered with pigments.

Reference:
WHO 2010 ; CDC Website

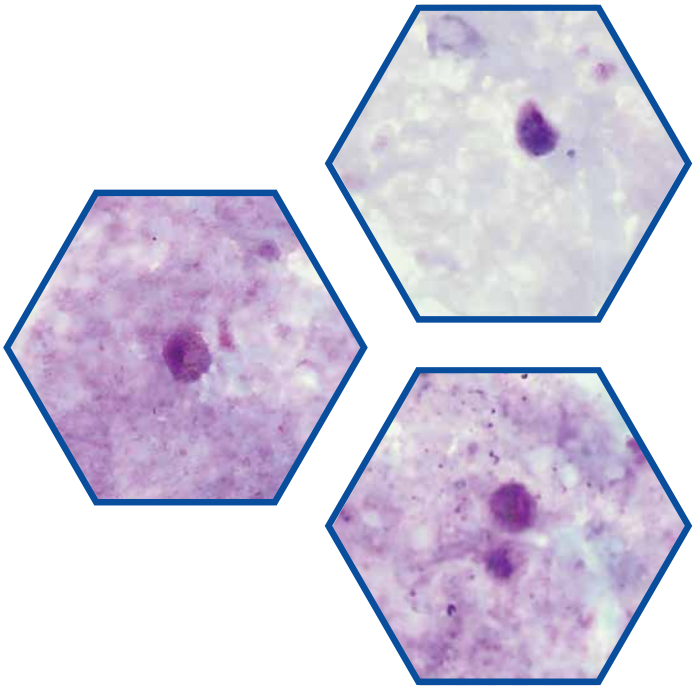
THICK BLOOD FILM
Plasmodium ovale



TROPHOZOITES
Trophozoites are enclosed within ghost cells. Ghost cells are observed as pinkish translucent cells containing the parasites. The chromatins are large and prominent while the cytoplasm is compact and dense.



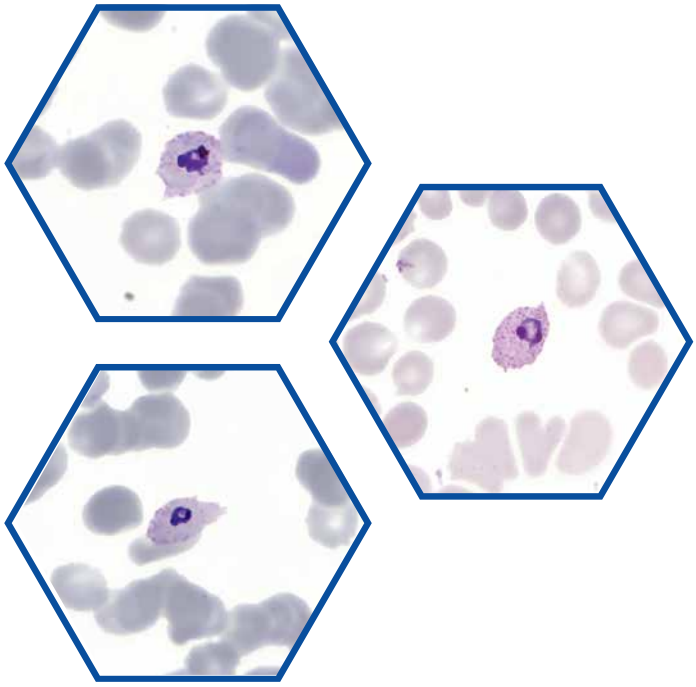
SCHIZONTS
Schizonts are known to have 4-12 merozoites in loose clusters accompanied by a mass of pigment.



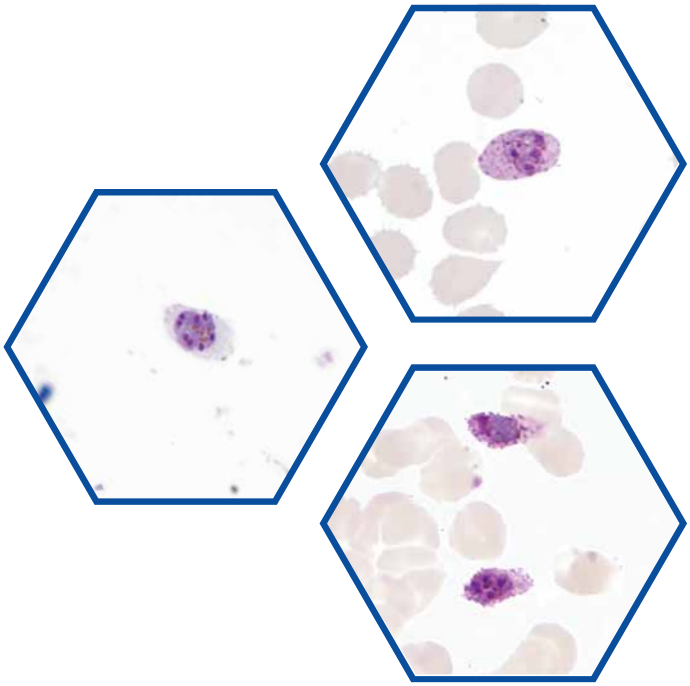
GAMETOCYTES
Gametocytes are round and compact with a single and enlarged chromatin. Pigments are scattered throughout the cytoplasm of the parasite.

Reference:
WHO 2010 ; CDC Website

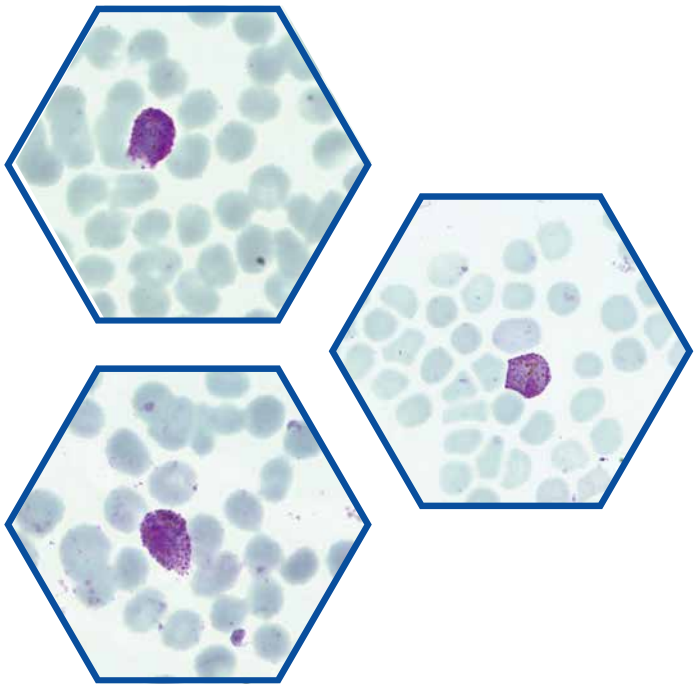
THIN BLOOD FILM
Plasmodium ovale



TROPHOZOITES
Infected RBCs are slightly enlarged and often described as oval-shaped or comet-shaped with fimbriations. Coarse stippling may also be observed on the infected RBCs. The chromatins of the parasites are large and prominent while the cytoplasm is compact and dense.



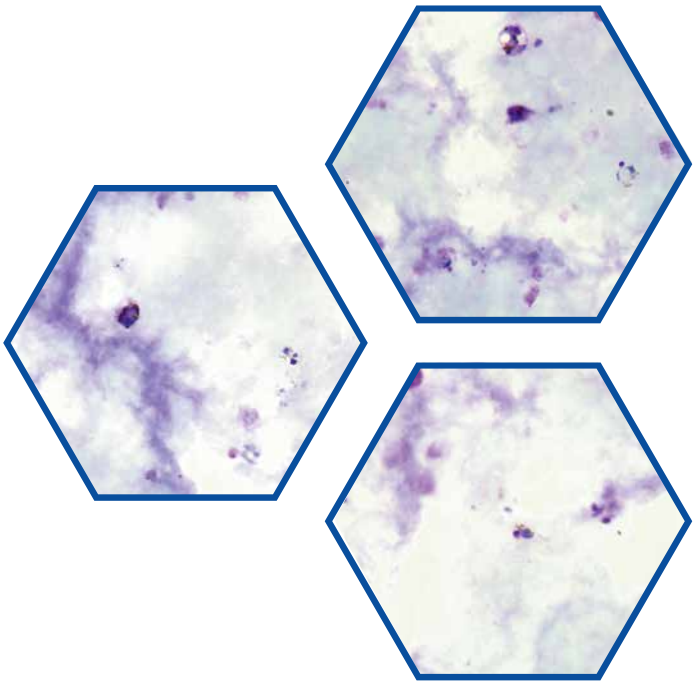
SCHIZONTS
Schizonts consist of 4-12 merozoites in loose clusters surrounding a mass of pigment. RBCs infected with schizonts may appear elongated with fimbriation.



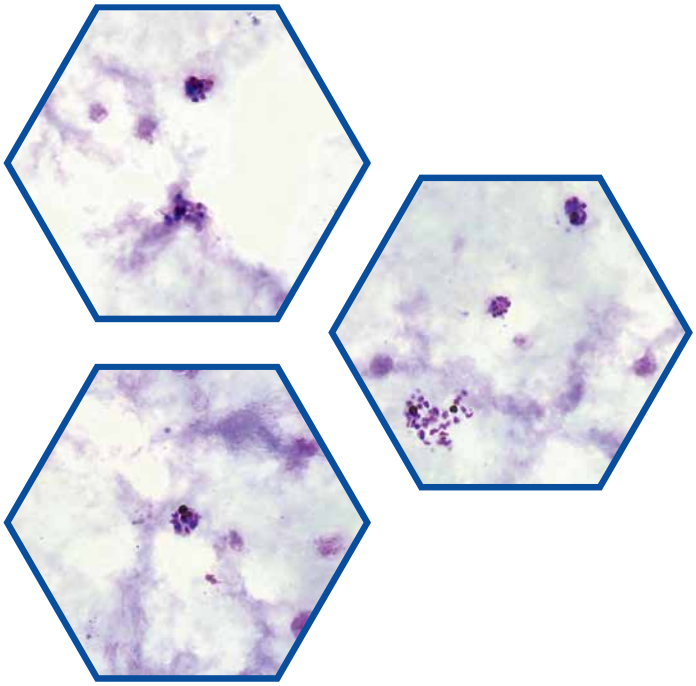
GAMETOCYTES
Gametocytes often fill the entire infected RBCs. The chromatin is enlarged and located eccentrically. The cytoplasm of the parasite is uniform and scattered with pigments.

Reference:
WHO 2010 ; CDC Website

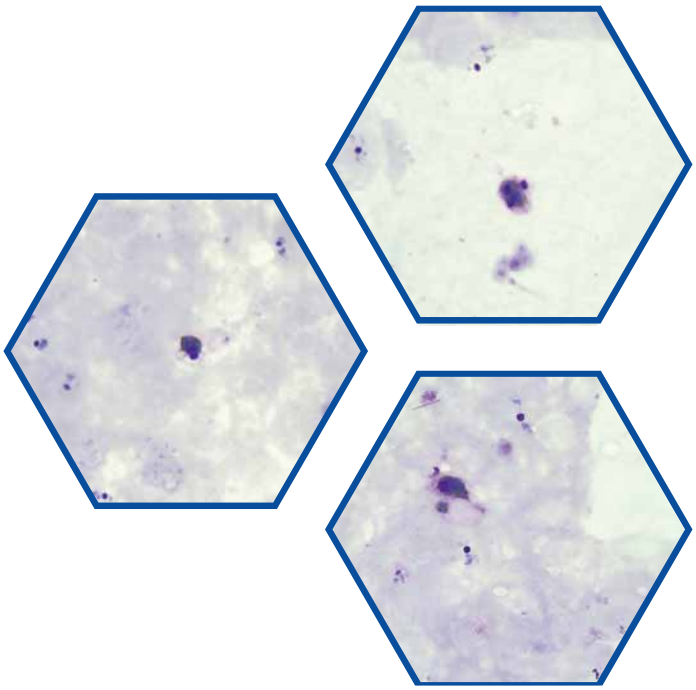
THICK BLOOD FILM
Plasmodium knowlesi



TROPHOZOITES
Unlike *P. falciparum*, all parasite stages of *P. knowlesi* in its erythrocytic cycle is commonly observed in the thick film. Trophozoites of *P. knowlesi* can be found in various shapes, often mimicking other human malaria parasites. However, trophozoites of *P. knowlesi* are commonly observed with dark brown to black pigment.



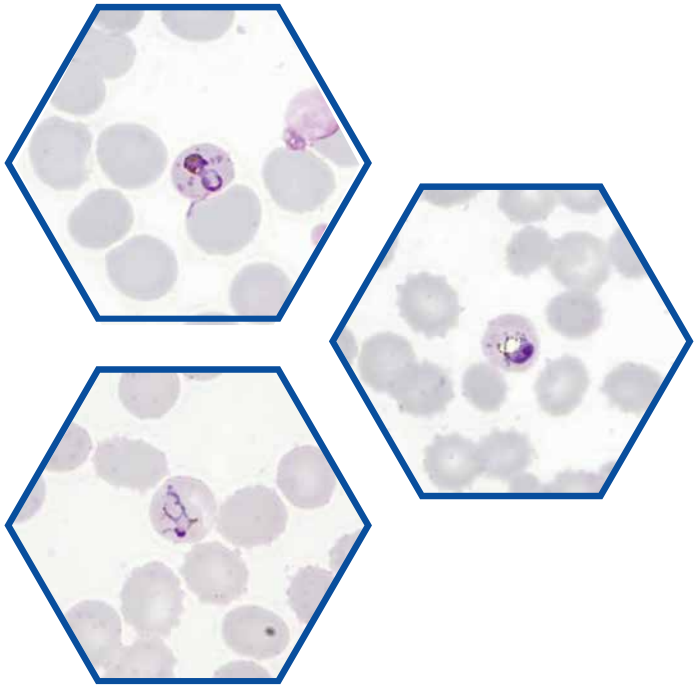
SCHIZONTS
Matured schizonts have up to 16 merozoites with large nuclei. The merozoites are clustered around a mass of coarse and dark brown to black pigment.



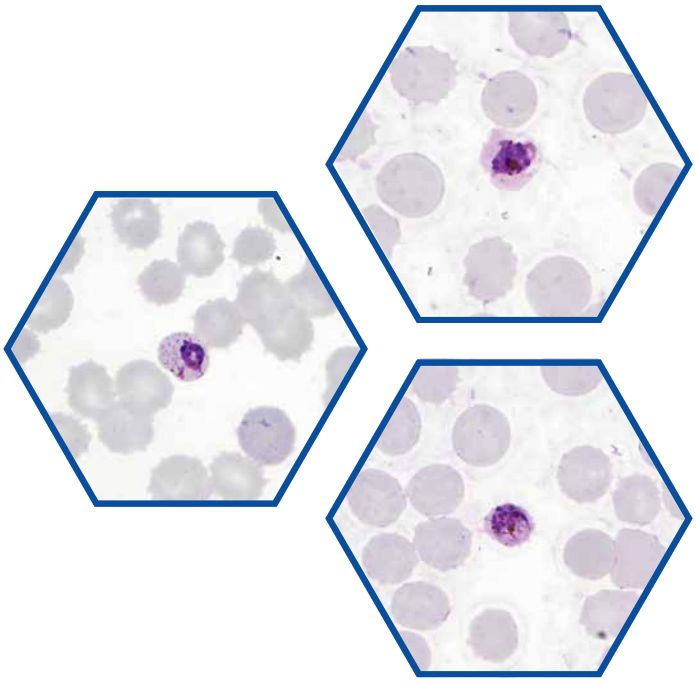
GAMETOCYTES
Gametocytes are commonly seen and appear round to oval shaped. Chromatin is compact and eccentric while the cytoplasm appears diffused. Dark brown to black pigments appear as scattered throughout the parasite.

Reference:
WHO 2010 ; CDC Website

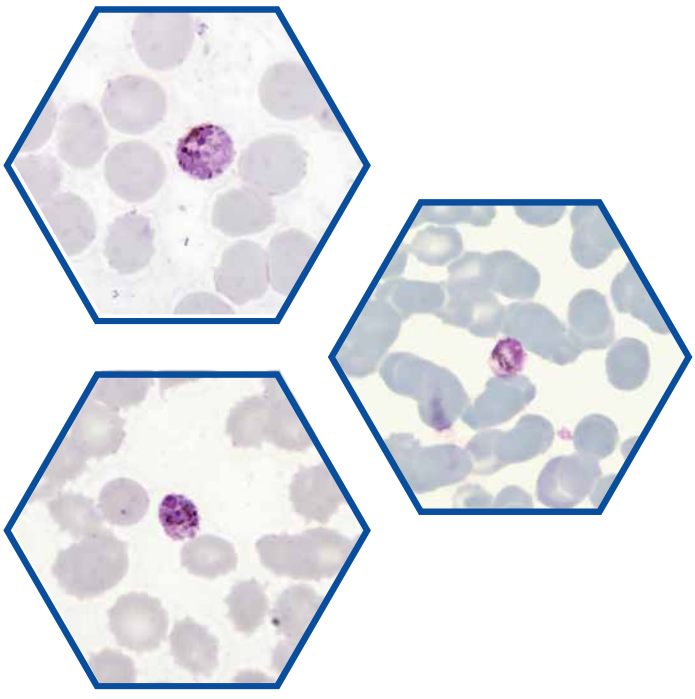
THIN BLOOD FILM
Plasmodium knowlesi



TROPHOZOITES
The infected RBCs are usually normal in size and occasionally exhibit stippling. Trophozoites are found in various irregular shapes and are commonly observed with dark brown to black pigments. Although the trophozoites of *P. knowlesi* mimic other trophozoites of human malaria parasites, the morphology is often not as well defined.



SCHIZONTS
Matured schizonts are observed to have about 16 merozoites with large nuclei. The merozoites are clustered around a mass of coarse, dark brown to black pigment.



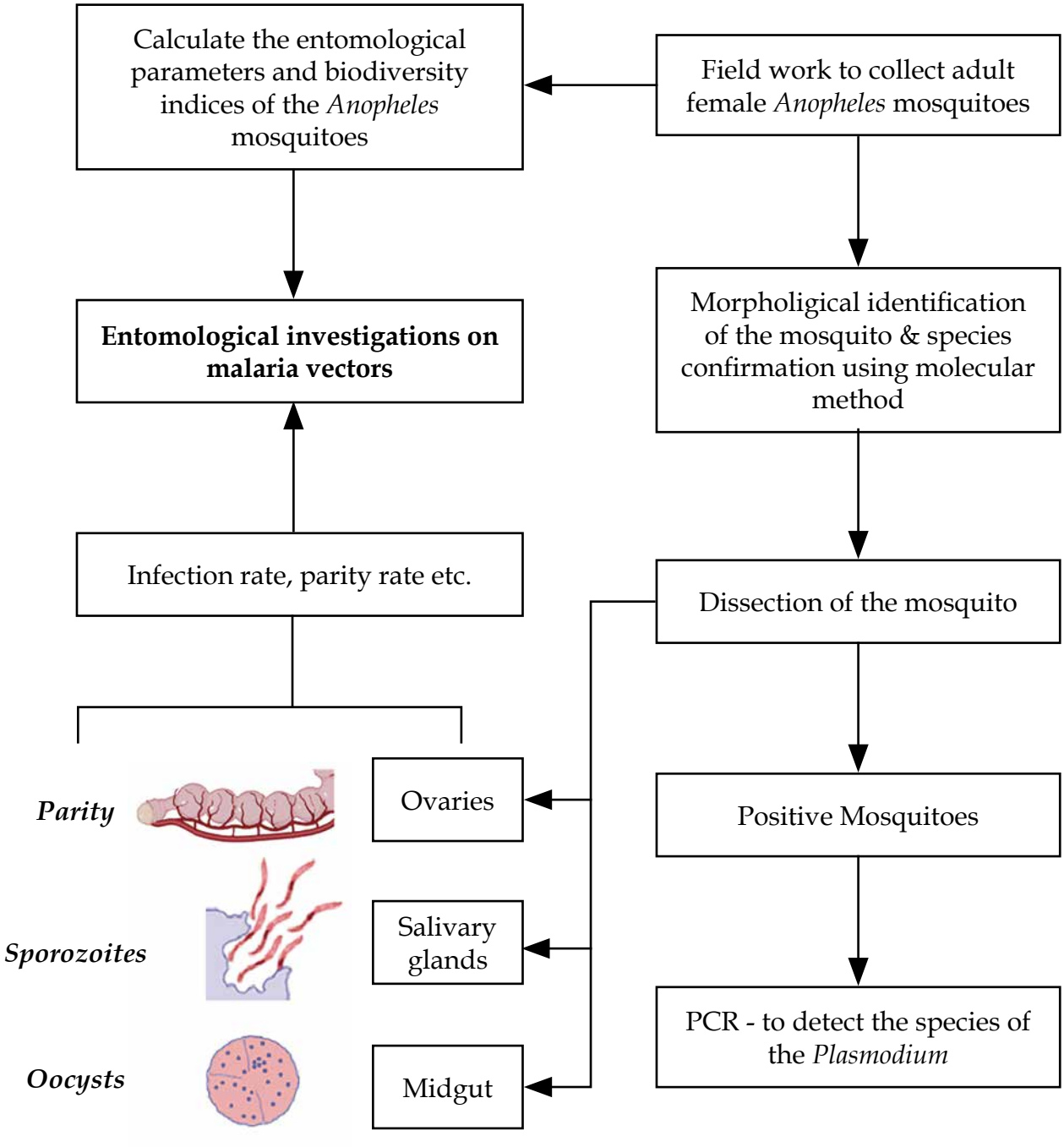
GAMETOCYTES
Gametocytes are spherical to oval shape and usually occupies most of the infected RBC. Chromatin is compact and eccentric. Dark brown to black pigment appear as irregularly scattered throughout the gametocyte.

Reference:
WHO 2010 ; CDC Website

Chapter 2

MALARIA VECTORS IN MALAYSIA

PROCESS FOR MALARIA VECTOR INCRIMINATION



MORPHOLOGICAL IDENTIFICATION OF MALAYSIA VECTORS

Identification key for adult anopheles (modified from Reid, 1968)

1. Maxillary palps usually about as long as the proboscis, abdomen without scales (Figure 1) .....***Anophelinae***

   Maxillary palps not more than two-thirds length of proboscis, abdomen entirely covered with scales above and below (Figure 2) .....***Culicinae***

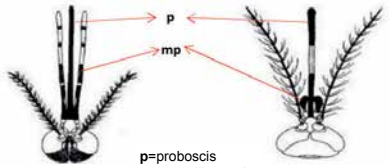


Figure 1 **p**=proboscis **mp**=maxillary palp; Figure 2

2. Wing commonly with less than 3 pale spots on the costa and vein 1, or costa all dark (Figure 3) .....subgenus ***Anopheles*****.....3**

   Wing commonly with 4 or more pale spots on the costa and vein 1 (Figure 4) ..... subgenus ***Cellia*** **12**

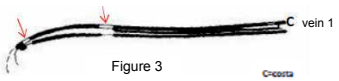


Figure 3

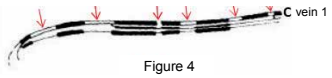


Figure 4

3. Hind femur without a distal broad white band, wing costa without presector pale spot (Figure 5)

. .....**4**

   Hind femur with a distal wide white band (either as an apical tuft of white scales, or a subapical pale band), wing costa with a well-marked presector pale spot (Figure 6).....**11**

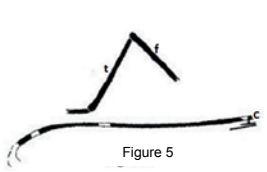


Figure 5

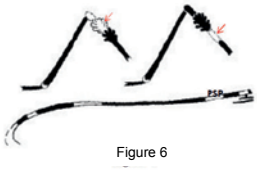


Figure 6

t=tibia; **f**=femur; **C**=costa; **PSP**=presector pale spot

4. Maxillary palps with pale bands (Figure 7).....**5**

   Maxillary palps without pale bands (males may have very narrow pale bands) (Figure 8)..... **6**

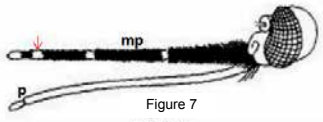


Figure 7

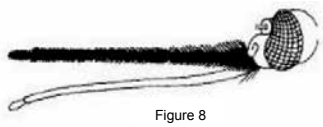


Figure 8

mp=maxillary palp; **p**=proboscis

5. Abdominal sternum with a small tuft of dark scales on the underside of segment VII, last segment of maxillary palps pale at apex, seldom entirely pale (Figure 9)....***hyrcanus sp.*** group

   Abdominal sternum without a tuft of dark scales on the underside of segment VII, last segment of maxillary palps usually entirely pale (Figure 10).....***separatus***

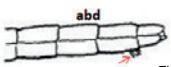


Figure 9

Figure 10

abd=abdomen; **mp**=maxillary palp

MORPHOLOGICAL IDENTIFICATION OF MALAYSIA VECTORS

Identification key for adult anopheles (modified from Reid, 1968)

6. Wings with light and dark areas (Figure 11) **7**
   Wings all dark (Figure 12) **9**

Figure 11

Figure 12

7. Hind tarsus 5 fully white (Figure 13) ..... ***albotaeniatus sp.*** group
   Hind tarsus 5 largely dark (Figure 14) **8**

Figure 13

Figure 14

8. Abdominal sternum with a tuft of dark scales on the underside of the segment VII, basal half of the wing costa has few scattered white scales (Figure 15) ..... ***barbirostris sp.*** group
   Abdominal sternum without any scales, basal half of the wing costa without scattered white scales (Figure 16) ..... ***umbrosus sp.*** group (less ***separatus*** and ***brevipalpis***)

Figure 15

Figure 16

W=wing; **C**=costa; **abd**=abdomen

9. Maxillary palps are as long as proboscis, head scales very narrow (Figure 17) .. ***aitkenii sp.*** group
   Maxillary palps shorter than proboscis, head scales broad (Figure 18) **10**

Figure 17

Figure 18

mp=maxillary palp; **p**=proboscis

10. Basal of fore femur swollen, thorax has several propleural setae on each side, pronotal lobes have scales (Figure 19) ..... ***brevipalpis***
    Basal of fore femur not swollen, thorax has 1 propleural setae on each side, pronotal lobes without scales (Figure 20) ..... ***sintenoides***

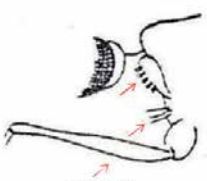


Figure 19

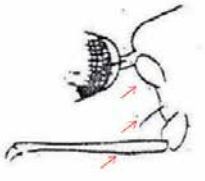


Figure 20

MORPHOLOGICAL IDENTIFICATION OF MALAYSIA VECTORS

Identification key for adult anopheles (modified from Reid, 1968)

11. Hind femur with a conspicuous tuft of white scales, preceded by a tuft of black scales (Figure 21)
. ..... ***asiaticus sp.*** group (no known medical importance)
Hind femur black, either smooth or tufted (femur with a subapical pale band), wing costa with presector pale spot (Figure 22) ..... ***lindesayi sp.*** group (no known medical importance)

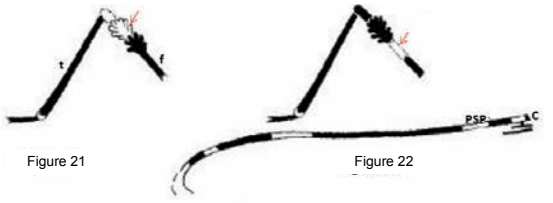


Figure 21 Figure 22

t=tibia; **f**=femur; **C**=costa; **PSP**=presector pale spot

12. Femur and tibia speckled with pale spots (Figure 23) **13**
Femur and tibia not speckled (Figure 24) **19**

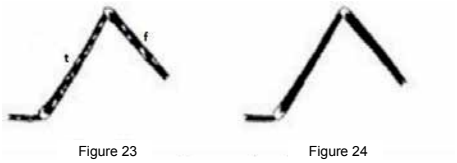


Figure 23 Figure 24

=tibia; **f**=femur

13. Hind leg with a broad white band at the tibio-tarsal joint (Figure 25) ***leucosphyrus sp.*** group
No broad white band at the tibio-tarsal joint of the hind leg (Figure 26) **14**

Figure 25 Figure 26

t=tibia; **ta**=tarsus

14. Maxillary palps with 3 pale bands, proboscis dark (Figure 27) **15**
Maxillary palps with 4 or more pale bands, outer half of proboscis may be pale (Figure 28) **17**

Figure 27 Figure 28

mp=maxillary palp; **p**=proboscis

15. Hind tarsus with narrow pale bands, tarsus 5 dark, coastal species (Figure 29)..... ***sundaicus***
Hind tarsus with broad white bands, tarsus 5 all white (Figure 30) **16**

Figure 29 Figure 30

MORPHOLOGICAL IDENTIFICATION OF MALAYSIA VECTORS

Identification key for adult anopheles (modified from Reid, 1968)

16. Last three segments of hind tarsus not all white, segments 4 and 3 each with a central dark band (Figure 31)..... ***maculatus***

Last three segments of hind tarsus all white (Figure 32)..... ***ramsayi***
jamesii

Figure 31

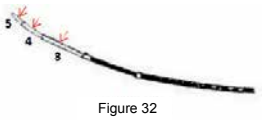


Figure 32

17. Underside of abdomen with tufts of black scales on segment II-VII (Figure 33)..... ***kochi***

No tufts of scales beneath the abdomen (Figure 34) **18**


Figure 33


Figure 34

18. Outer half of proboscis pale, last three segments of hind tarsus dark with narrow pale band (Figure 35)..... ***tessellatus***

Proboscis dark, last three segments of hind tarsus or at least the third segment largely white (Figure 36)..... ***watsonii***
stookesi
saungi

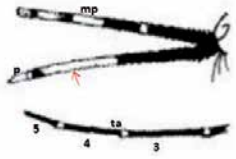


Figure 35

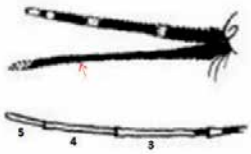


Figure 36

mp=maxillary palp; **p**=proboscis; **ta**=tarsus

19. Hind tarsus 5 all white (Figure 37) **20**

Hind tarsus 5 dark or partly dark (Figure 38)..... **21**

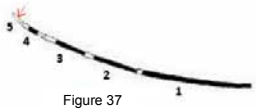


Figure 37

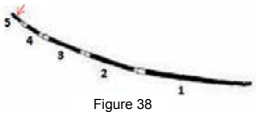


Figure 38

20. Female maxillary palps with 4 pale bands, only the fifth hind-tarsal segment all white (Figure 39)..... ***karwari***

Female maxillary palps with 3 pale bands, hind-tarsal segments 5, 4 and 3 all white (Figure 40) ..... ***annularis sp.*** group

Figure 39

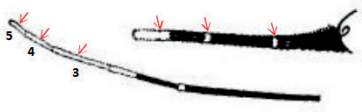


Figure 40

mp=maxillary palp; **p**=proboscis; **ta**=tarsus

MORPHOLOGICAL IDENTIFICATION OF MALAYSIA VECTORS

Identification key for adult anopheles (modified from Reid, 1968)

21. Fore tarsus with narrow pale bands or unbanded (Figure 41) **22**
Fore tarsus with broad pale bands (Figure 42) **24**

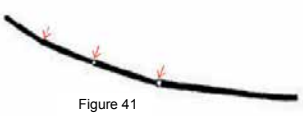


Figure 41

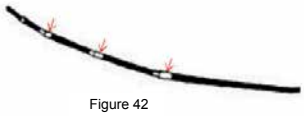


Figure 42

22. Maxillary palps with not more than 3 pale bands, hind margin of the wing with pale fringe spots at the ends of the veins (Figure 43) **23**
Maxillary palps with 4 pale bands, hind margins of the wing without pale fringe spots at the ends of vein end (Figure 44) ..... ***aurirostris***

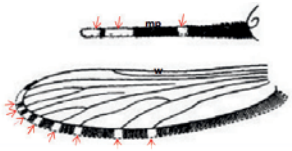


Figure 43

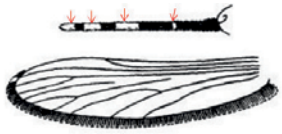


Figure 44

mp=maxillary palp; **p**=proboscis

23. Outer half of the proboscis all pale, wing with a fringe spot at end of vein 1A (Figure 45) . ..... ***aconitus***
Outer half of the proboscis dark, wing often without a fringe spot at vein 1A (Figure 46) . ..... ***minimus***

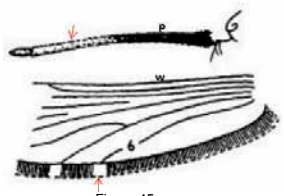


Figure 45

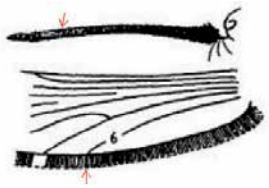


Figure 46

p=Ptobostis; **w**=wing

24. Maxillary palps with preapical dark band, usually a quarter or less as long as apical pale band, proboscis with a pale mark towards the end (Figure 47) ..... ***vaoftus***
Maxillary palps with preapical dark band, usually a third or more as long as apical pale band, proboscis usually dark (Figure 48) ..... ***subpictus indefinitus***

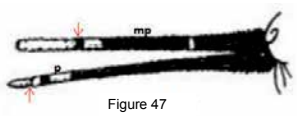


Figure 47

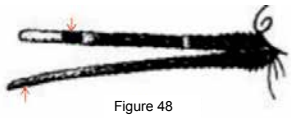


Figure 48

mp=maxillary palp; **p**=proboscis

MORPHOLOGICAL IDENTIFICATION OF MALAYSIA VECTORS

Identification key for adult Leucosphyrus Group of mosquitoes, modified from Sallum et al., 2005 (Jeffrey et al. 2012)

1. Proboscis always much longer than maxillary palpus, longer than forefemur (Figure 49)....**2**

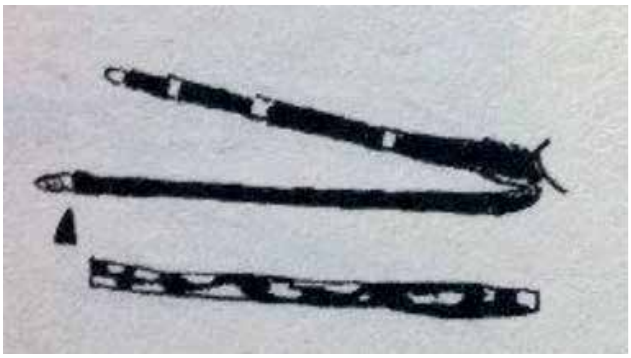


Figure 49

Proboscis at most slightly longer than maxillary palpus, as long as or slightly longer than forefemur (Figure 50).....**3**

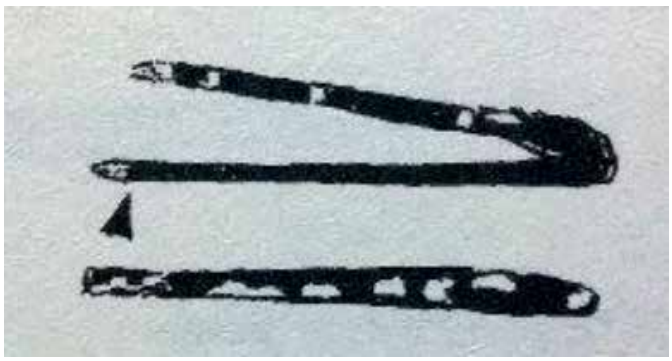


Figure 50

2. Apical pale band of palpomere 5 very narrow, proboscis is dark with narrow apical ring basal to labella (Figure 51) .....***hackeri***

Figure 51

Apical pale band of palpomere 5 very broad, proboscis has pale scales at apex basal to labella (Figure 52).....***pujutensis***

Figure 52

MORPHOLOGICAL IDENTIFICATION OF MALAYSIA VECTORS

Identification key for adult Leucosphyrus Group of mosquitoes, modified from Sallum et al., 2005 (Jeffrey et al. 2012)

3. Accessory sector pale (ASP) spot usually extending onto costa (C) and subcostal (SC) at least on one wing (Figure 53 ..... **4**

Figure 53

ASP spot absent on costa and usually absent on subcostal (Figure 54) **5**

Figure 54

4. Hindtarsomere 4 without obvious basal pale band on dorsal surface (Figure 55)..... **6**

Figure 55

Hindtarsomere 4 with patch of pale scales on dorsal surface basally (Figure 56)..... ***balabacensis***

Figure 56

5. Presector dark (PSD) spot on vein R without pale interruptions (Figure 57) ..... ***macarthuri***

Figure 57

PSD spot on vein R with or without pale interruptions (Figure 58)..... **7**

Figure 58

MORPHOLOGICAL IDENTIFICATION OF MALAYSIA VECTORS

Identification key for adult Leucosphyrus Group of mosquitoes, modified from Sallum et al., 2005 (Jeffrey et al. 2012)

6. PSD spot of vein R often not extending basally beyond PSD spot on costa and rarely extending onto apical ½ of humeral dark (HD) spot on costa, apical pale band of palpomere 5 light cream-colored, not strongly contrasting with pale bands on palpomere 2 and 3 (Figure 59) ..... ***introlatus***

Figure 59

PSD spot of vein R often not extending basally onto level of HD on costa or beyond middle of HD, apical pale band of palpomere 5 distinctly cream-colored, strongly contrasting with silvery white band on palpomere 2 and 3 (Figure 60) ..... ***latens***

Figure 60

7. All pale scales of wing cream-colored to golden to yellowish, including presector pale (PSP) and sector pale (SP) spots, PSD spot of vein R often not extending basally beyond level of PSD wing spot on vein C (Figure 61) ..... ***nemophilous***

Figure 61

All pale scales on anterior veins of wings (including PSP and SP on costa) white, contrasting with pale spots on posterior, PSD spot of vein R frequently extending basally beyond ½ of PSP but not beyond HD, sternum VI with a small posteromedial patch of dark scales (Figure 62) ..... ***cracens***

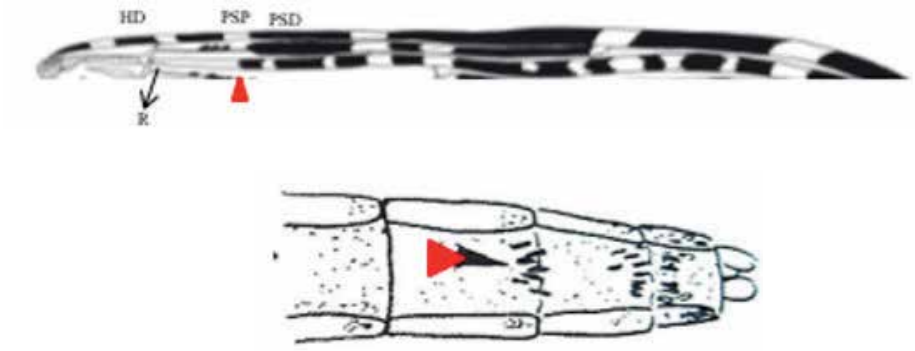
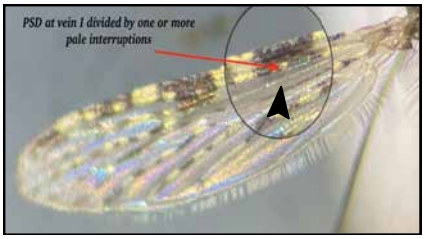


Figure 62

MORPHOLOGICAL FEATURES: GUIDE FOR COMMON SPECIES OF MALARIA VECTORS IN MALAYSIA (LEUCOSPHYRUS GROUP)

Anopheles balabacensis

Hind leg with broad white band at tibio-tarsal joint

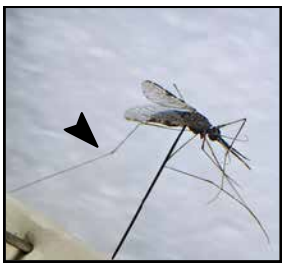


PSD at vein 1 dividedby one or more paleinterruptions

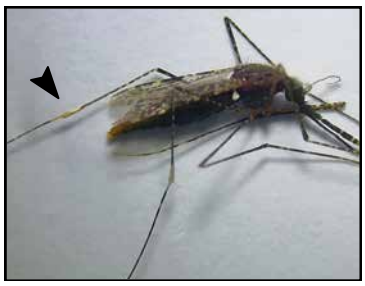
Length of proboscis equal with fore femur

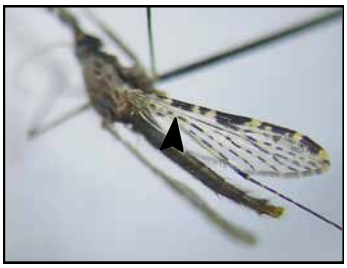
Hind tibia: Apical pale band without a ventral dark stripe

Anopheles cracens



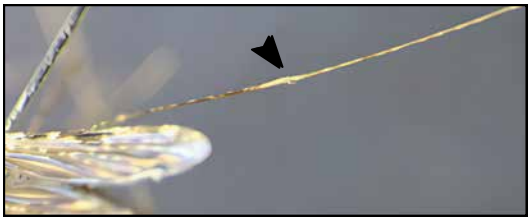
Hind leg with broad white band at tibio-tarsal joint



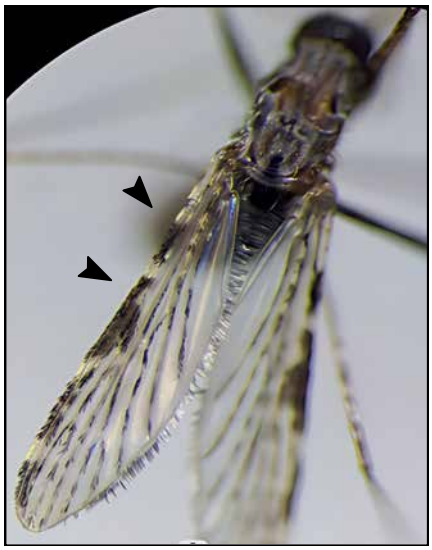


PSD on vein R with pale interruption

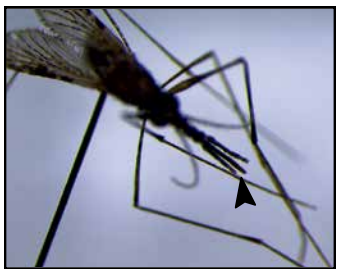
Abdominal sternum VI with small posteriomedial patch of dark scales



Hind tibia: Apical pale band without a ventral dark stripe



- PSD spot on vein R with pale interruption
- Pale scales on anterior wings white, contrasting pale spots on posterior
- PSD spot on vein R extending basally beyond ½ of PSP but not beyond HD
- ASP spot absent on costa and subcostal

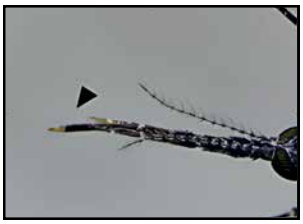


Proboscis most slightlylonger than maxillary palpus and fore femur

MORPHOLOGICAL FEATURES: GUIDE FOR COMMON SPECIES OF MALARIA VECTORS IN MALAYSIA (LEUCOSPHYRUS GROUP)

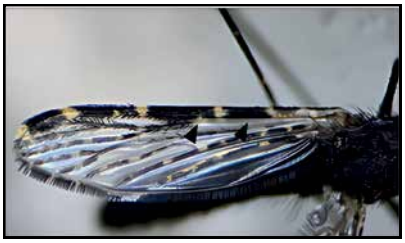
Anopheles hackeri

Tibio-tarsal joint of hind leg with large white band



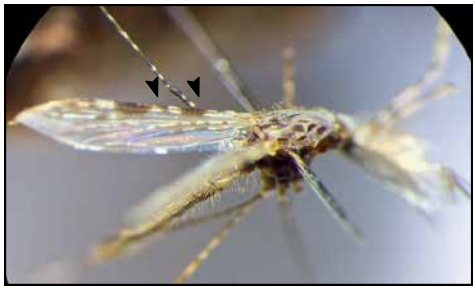
Proboscis distinctly longer than fore femur (ratio 1.16-1.45) and usually much longer than maxillary palpus, often with narrow pale band or patch before labella

Hind tibia with or without narrow apical extension of dark scales on ventral aspect

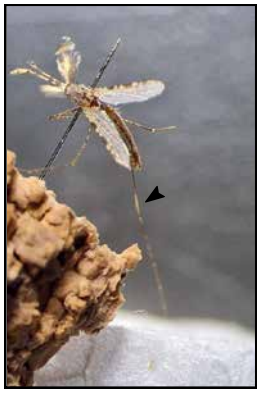


- Presector dark (PSD) spot of vein R variable with or without pale interruption
- Accessory sector pale (ASP) spot very small and restricted to vein R, shorter than basal dark spot of sector dark (SD) of vein R and sometimes reduced to 1 or 2 scales

Anopheles introlatus



- PSD spot of vein R not extending basally beyond PSD spot on cost
- ASP spot extending onto costa and subcostal

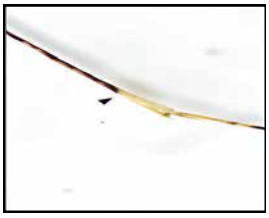


Hind leg with broad white band at tibio-tarsal joint

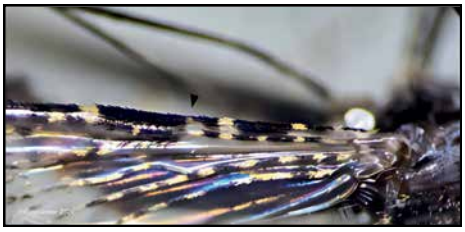
Proboscis slightly longer than maxillary palpus and fore femur

Anopheles latens

Maxillary palpus: Palpomere 5 distinctly cream colour, contrasting with silvery-white bands on Palpomere 2,3

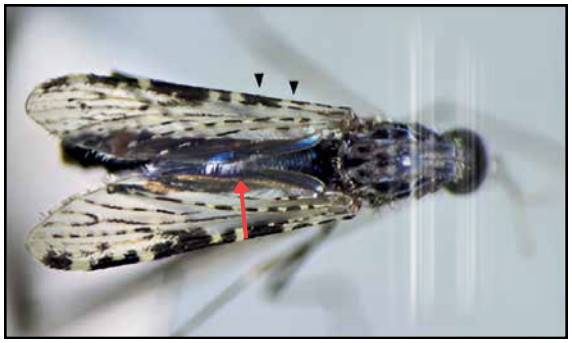


- Tibio-tarsal joint of hind leg with large white band
- Hind tibia without narrow apical extension of dark scales on ventral sides



Accessory sector pale (ASP)spot usually extending on to subcosta and costa at least on one wing

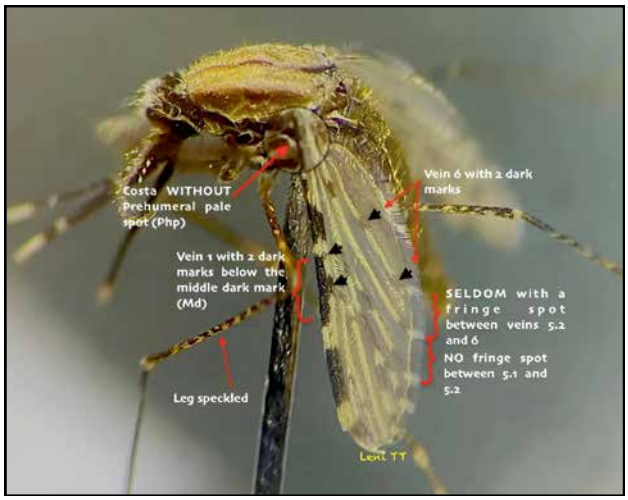
- Proboscis shorter or only slightly longer than fore femur (ratio 0.88-1.17) and only slightly longer than maxillary palpus
- Proboscis without pale scales on apical half

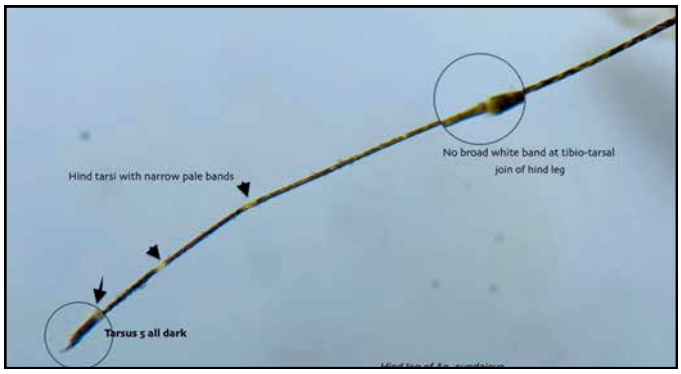


Presector dark (PSD) spot of vein R usually extending basally well onto level of humeral dark (HD) spot of costa, or beyond middle of HD (levels 4 and 5)

MORPHOLOGICAL FEATURES: GUIDE FOR COMMON SPECIES OF MALARIA VECTORS IN MALAYSIA (LEUCOSPHYRUS GROUP)

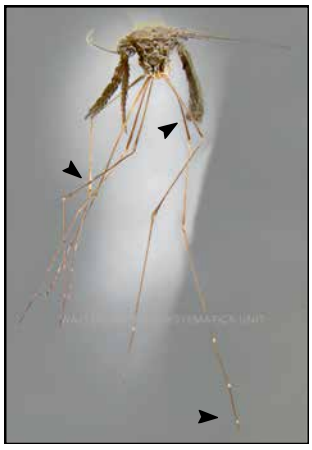
Anopheles epiroticus





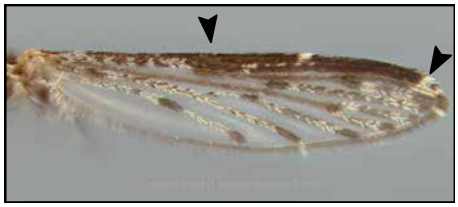
Hind tarsi with narrow white rings. Tarsus 5 black

Anopheles donaldi



First pale band on Ta-I about as long as Ta-I5. Fe-III without a preapical paleband; Ta-II1–5 with narrow apical pale bands.

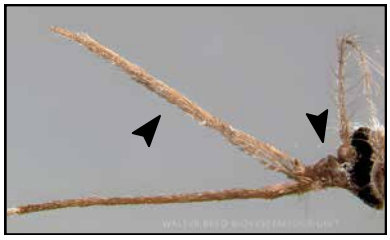
I–VI-S each with ≥20 median pale scales; VII-S with tuft of dark scales.

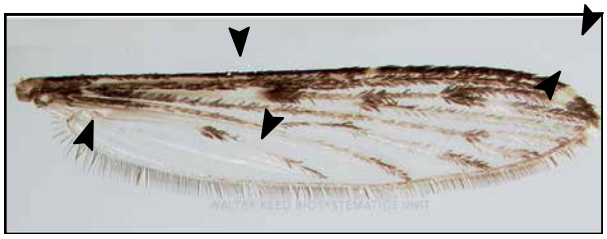


Costa without presector pale spotor preapical pale spot; wing apex with 3 small pale spots, center spot near end of vein R2, infrequently with 2 or 3 dark scales near base.

Anopheles letifer

Palpus unicolorous; clypeus without lateral patches of dark scales



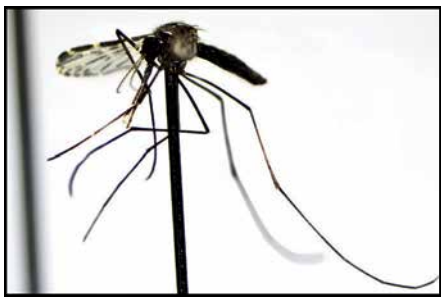


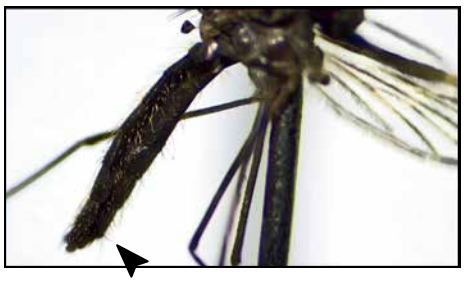
Costa without presector pale spot; base of veins Rand CuA with dark scales; wing apex with 2 pale spots, without pale spot at vein R2; vein 1A pale-scaled proximal to median

- Source of photos *Anopheles donaldi* (WRBU, 202a)
- Source of photos *Anopheles letifer* (WRBU, 2021b)

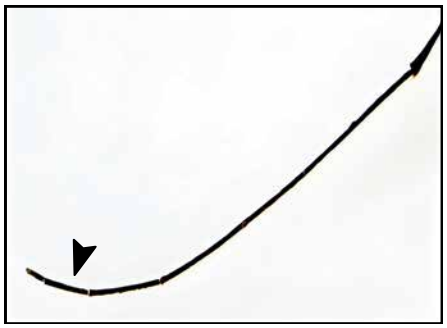
MORPHOLOGICAL FEATURES: GUIDE FOR COMMON SPECIES OF MALARIA VECTORS IN MALAYSIA (LEUCOSPHYRUS GROUP)

Anopheles flavirostis

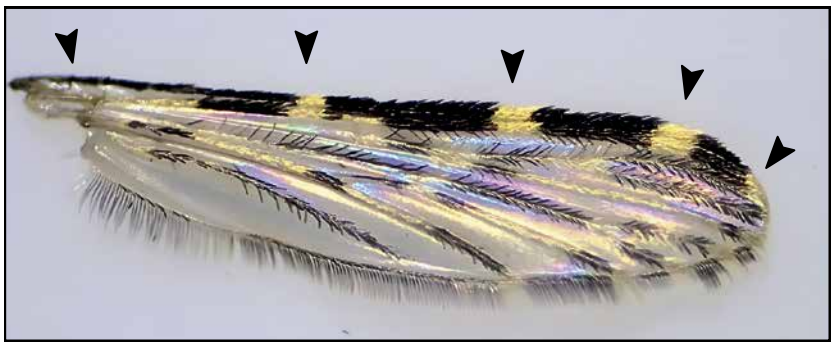




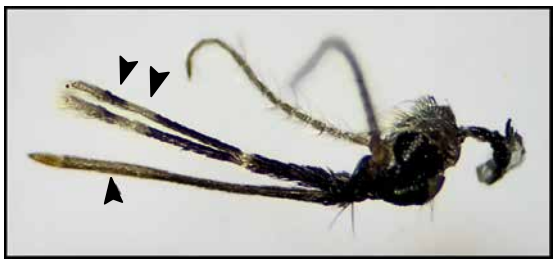
Abdominal sternites II-VI without tufts of dark scales



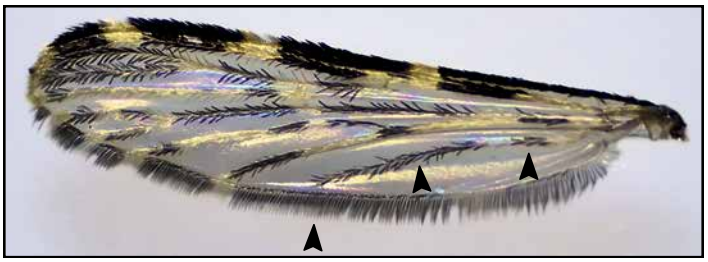
Last tarsomere of hind legs either entirely, or at least partially, dark scaled



- Basal third of costal vein usually dark, or with only one pale spot present
- Costal vein of wing with four or more pale spots, sector pale spot always present
- Fork of wing vein Cu with dark scales



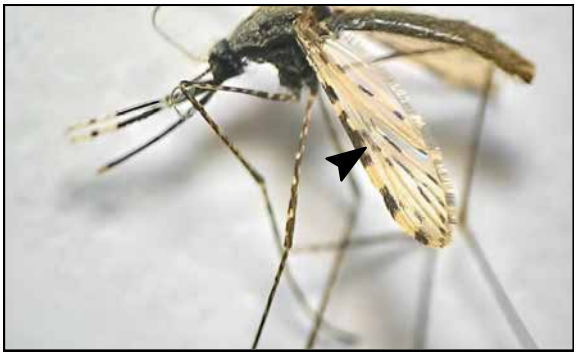
- Subapical dark band of palpi shorter than subapical pale band
- Proboscis with ventral golden patch, usually confined to apical half



Wing vein A with only two dark areas, distal one long, basal one short, fringe opposite this vein dark

Anopheles maculatus

Leg speckled



Veins C and R–R1 with ≥4 dark spots

Hind tarsomere 5 entirely white

DISSECTION OF ADULT FEMALE MOSQUITOES FOR MALARIA PARASITES

1 For each mosquito prepare two slides. One with two drops of saline and the other with a drop of mercurochrome and a drop saline.
2. The adult mosquito is killed by freezing or placing a drop of killing mixture or chloroform.
3. Remove legs and wings.
4. Place on a slide containing two drops of saline.
5. The mosquito abdomen should be towards the right-hand side. Hold the thorax region with one needle (in the left hand) and with the other needle gently pull at the abdomen so that the midgut and ovaries are removed from the body cavity of the mosquito.
6. The midgut is recognised by the presence of the Malpighian tubules.
7. The midgut is then detached, lifted with the needle and dropped into the mercurochrome on the other slide. Place a cover slip over it.
8. Transfer the head and thorax to the 2nd drop of saline. Exert gentle pressure upon the thorax with the needle in the left hand so that the neck membrane is somewhat distended by the pressure of the fluid within the mosquito.
9. With the second needle in the right hand pull the head towards the right maintaining pressure on the thorax at the same time until the salivary glands are completely out from the thorax.
10. Cut and transfer the salivary glands (3 lobes) to the drop of saline and place the cover slip over it.
11. The slide must be kept in a moist chamber until examination.

Examination of the midgut.

1. Examine the midgut under low power and confirm under high power.
2. Oocysts are only found on the outer surface of the gut.
3. Young oocyst are clear oval round bodies containing distinct pigment granules.
4. The number of oocysts vary. Press on the cover slip to ensure it is oocyst. Sometimes artifacts are seen and will move away when pressed. If oocyst will remain in position.
5. If positive transfer midgut to Eppendorf tube containing 90% alcohol to carry out PCR

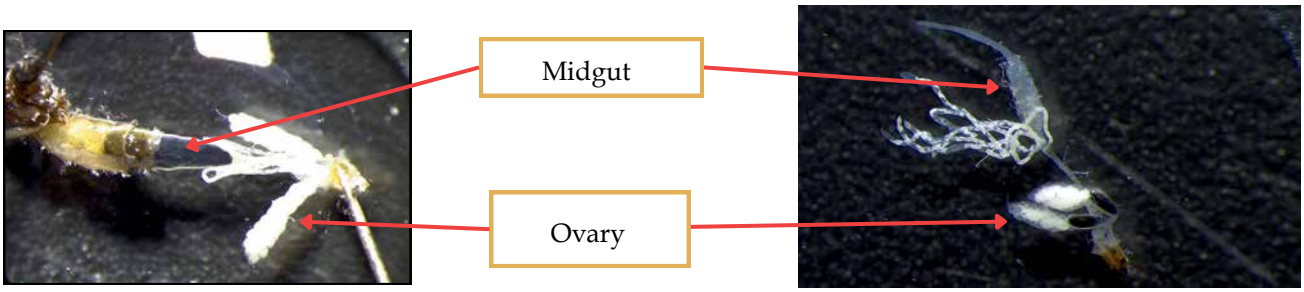
Examination of salivary gland

1. Examine under low power and later under high power. 2. Sporozoites are not visible in intact glands.
3. Press of the cover slip gently with the wooden stick to rupture the salivary gland.
4. If positive sporozoites will be seen swimming around. They are spindle shaped structures.
5. If positive transfer salivary gland to Eppendorf tube containing 90% alcohol to carry out PCR.
6. Transfer the remaining salivary glands (3 lobes) into another Eppendorf tube containing 90% alcohol to carry out PCR.
7. * For PCR refer to Jeyaprakasam & Vythilingam 2024

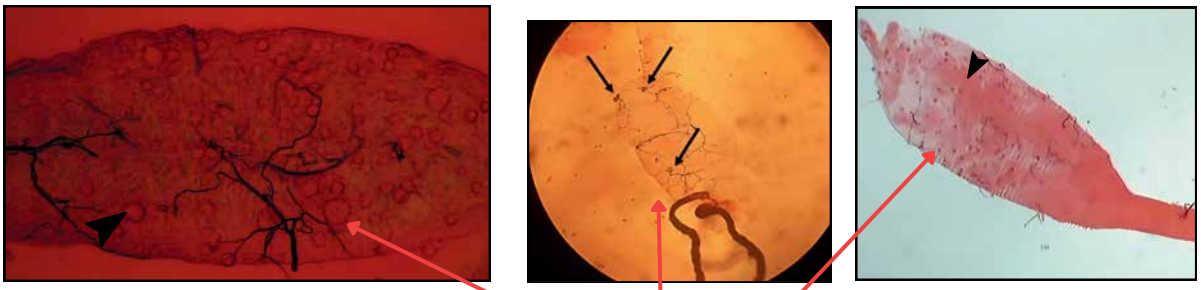
Source: Vythilingam, I.

DISSECTION OF ADULT FEMALE MOSQUITOES FOR MALARIA PARASITES

Dissection to obtain midgut

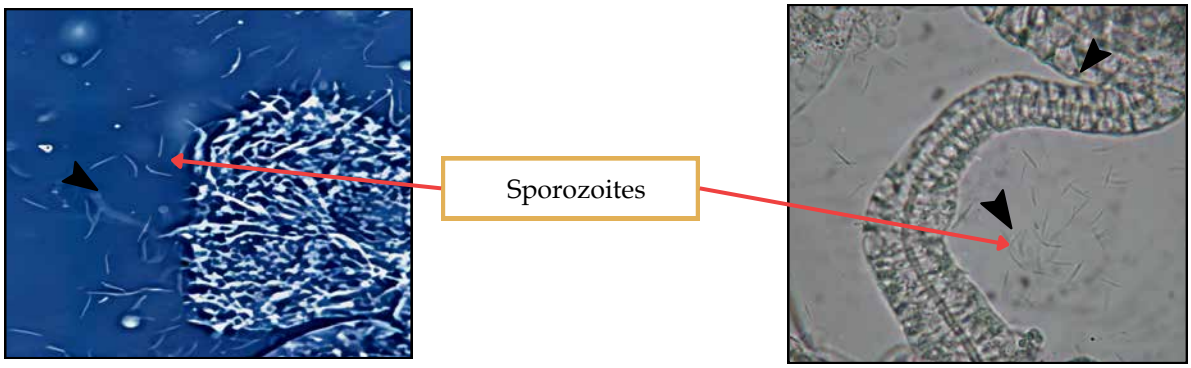


Oocycts on midgut



Dissection to obtain salivary gland

Sporozoites in salivary gland



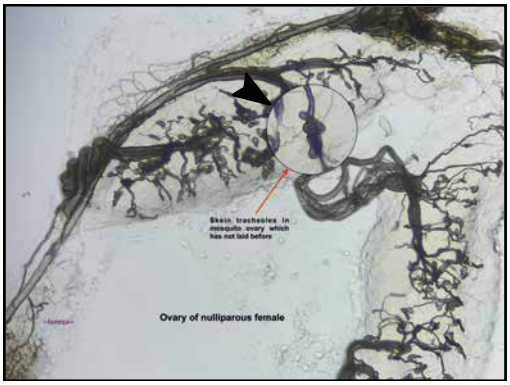
DISSECTION OF ADULT FEMALE MOSQUITOES FOR PARITY STATUS

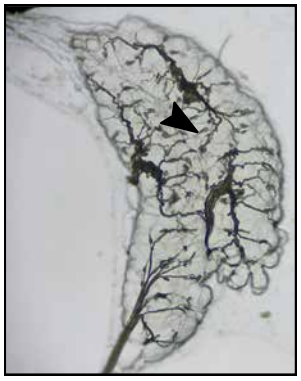
Examination of the ovary

1. Mosquito is killed with a drop of chloroform or killing mixture.
2. Remove the wings and legs by using dissecting needles or with your fingers.
3. Place two drops of saline on a clean glass slide and then place the specimen over the central drop of saline.
4. With the needle in the left hand put some pressure on the thorax and with the second needle placed at the tip of the abdominal segment pull out gently the last 2 segments hanging from which should be two ovaries. Transfer one ovary on to a clean labelled dry slide without saline and allow to dry. This is to check if the mosquito is parous or nulliparous.
5. Observe the tracheolar coiling in the ovary under compound microscope (10X) to check if the mosquito is parous or nulliparous.

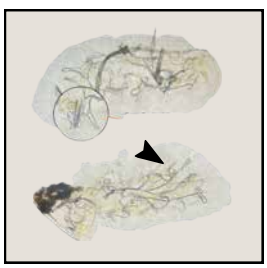
Source: Vythilingam, I.

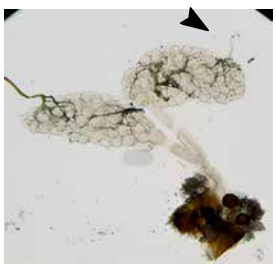
Dissection to obtain ovaries of mosquito

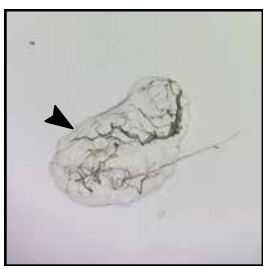




Nulliparous female
Terminal ends of the tracheoles are tightly coiled into Skeins.







Parous female
Tracheoles are stretched and uncoiled.

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ISBN 978-967-13278-5-2

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