

Mountain White Crowned Sparrows (MWCS) Blood Smear Scoring Protocol

Background

The white-crowned sparrow project has been going on since the early 2000s, in the lab an important aspect of the project is the scanning for avian malaria parasites in the blood smears taken during the field season (for more information on what happens during the field season check out the [Methods Protocol for the Mountain White-crowned sparrow project 2005](#) document in the lab, this might help in understanding the scope of the project). The following is taken from that protocol:

The larger goal of this study is to examine how avian wildlife handle different selective pressures and what tradeoffs they make during the breeding season. We are particularly interested in how food resources, parasites, and human disturbance interact to influence reproductive success in White-crowned Sparrows (*Zonotrichia leucophrys oriantha*). In addition, we want to explore some of the basic transmission dynamics of avian haemosporidian parasites found in the sparrow population. Haemosporidian parasites are protozoan blood parasites (i.e. malaria) that are transmitted by dipteran vectors, such as mosquitoes, black flies and biting midges.

How to Count Smears

Standard protocol for determining parasitemia levels (intensity of parasite infection) is to count parasites per 10,000 red blood cells (RBCs). However it is not practical to count 10,000 RBCs on every blood smear. All blood smears are scored at 100x using oil emersion.

Therefore count the number of RBCs in 1/8 of the field! Work in a zig-zag motion through out the slide until you reach a count of 1250 RBCs (the counter will zero-out at 1000 RBCs and continue on to 250) then multiply by eight.

On each field that you are counting, count EVERY parasite in the entire field, not just parasites located in the 1/8 of the field that you are counting RBCs.

In the drawing above the Xs are parasites, therefore you would count all four parasites. Even though you are counting 1/8 of the field. There are no gridlines on the microscope field to determine 1/8 of the field and it just takes practice.

Counting only 1/8 of fields that have parasites, this will not give an accurate representation of parasite levels, as there may not be any parasites for 10,000 RBCs.

Also making a count of 1/8 of the field that represents the rest of the field, that is it is not overly precise. It is like the other 7/8 of the field.



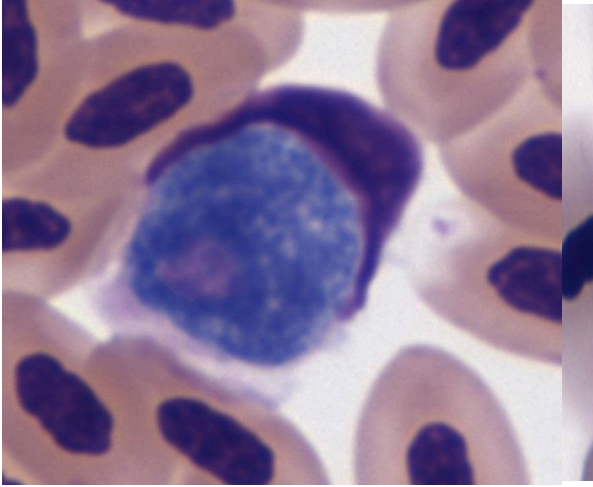
The Parasites

We look for 4 different genera of parasites and 6 different species. Macro and Micro parasites are the gametocytes of the parasite and involved in reproduction with in the vector. Macro parasites are stained in a blue color while Micro parasites are stained in a light pinkish purple.

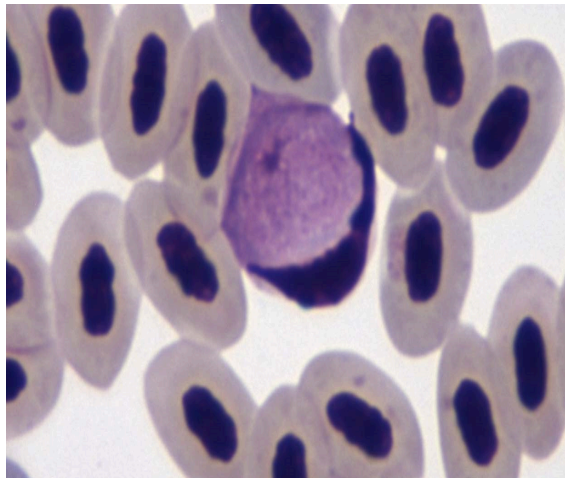
Malarian parasites:

Leucocytozoon, vectored by blood-feeding Simuliid flies (Diptera), results in the development of gigantic cell accumulations of immature stages (megalmegameres) in pectoral muscle, spleen, liver, lungs, heart, brain, and internal organs. The two species we

look for are *Leucocytozoon fringillinarum* and *Leucocytozoon majoris*. *Leucocytozoon* are generally round in shape.
Leucocytozoon fringillinarum: *L. fringillinarum* is characterized by pushing the nucleus into a tip.

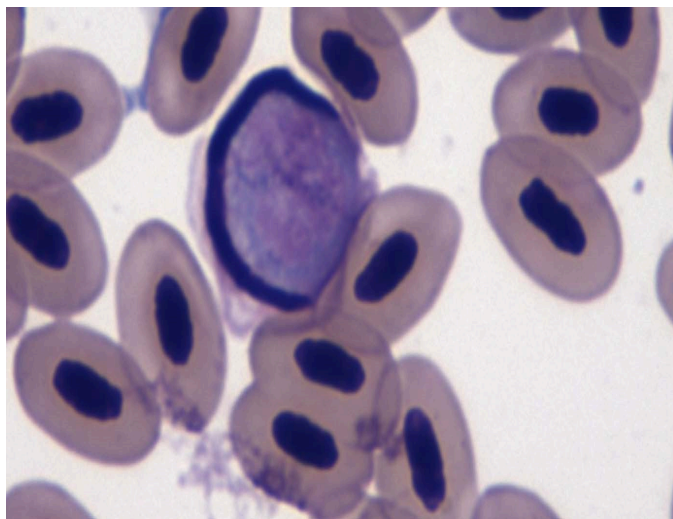
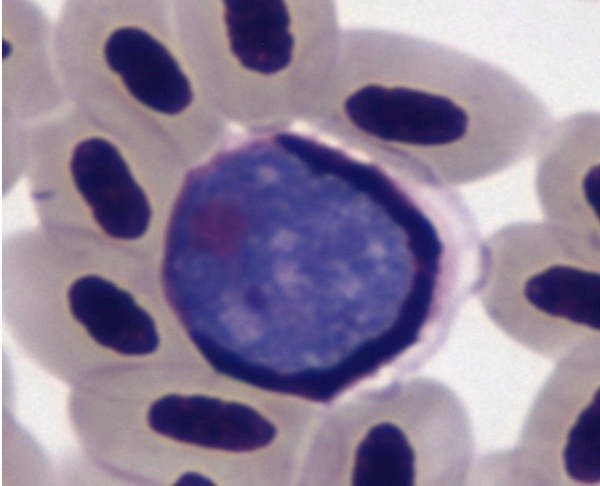


Macro



Micro

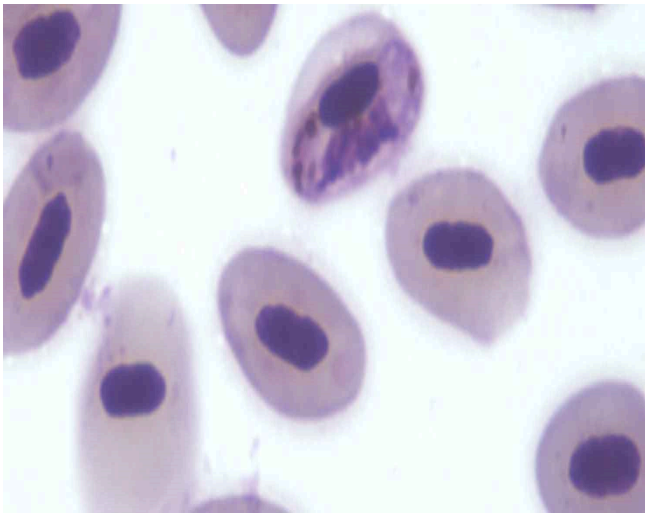
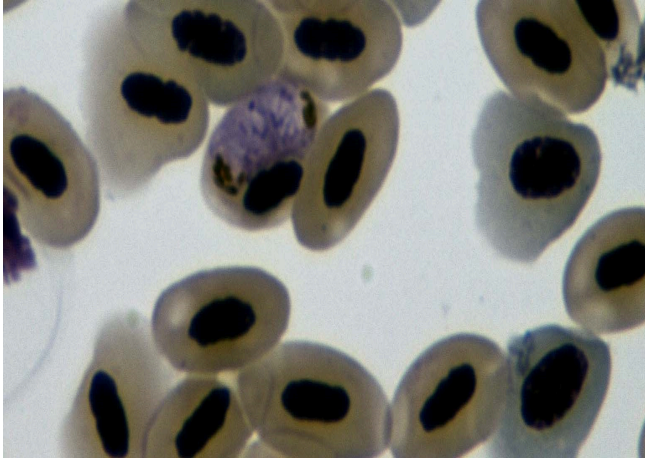
Leucocytozoon majoris: *L. majoris* is characterized by pushing the nucleus into a virtual ring around the parasites.



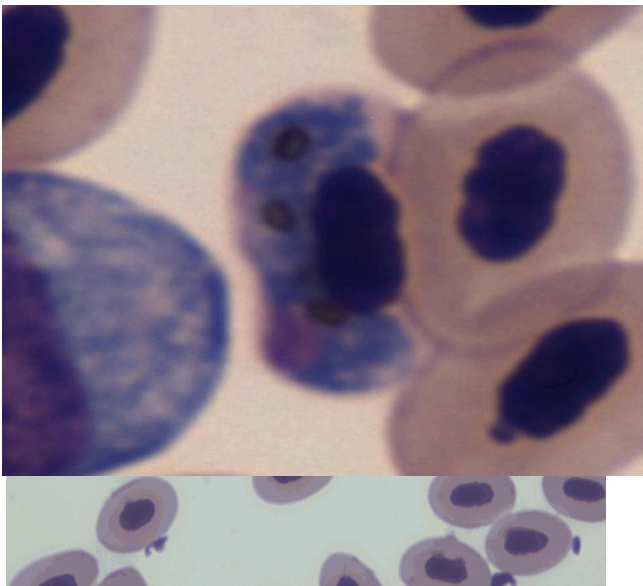
Micro

Note: *Leucocytozoon* is the largest and the easiest to identify. Also note the translucent film around the parasite, this is also a key identifier!

Haemoproteus, vectored by biting midges (Diptera: Ceratopogonidae) and hippoboscids (Diptera: Hippoboscidae), can also cause the destruction of red blood cells, resulting in anemia. *Haemoproteus coatneyi* is the species found in MWCS it is distinguished by its hand/bar bell shape and yellowish granules within the parasite.



Micro

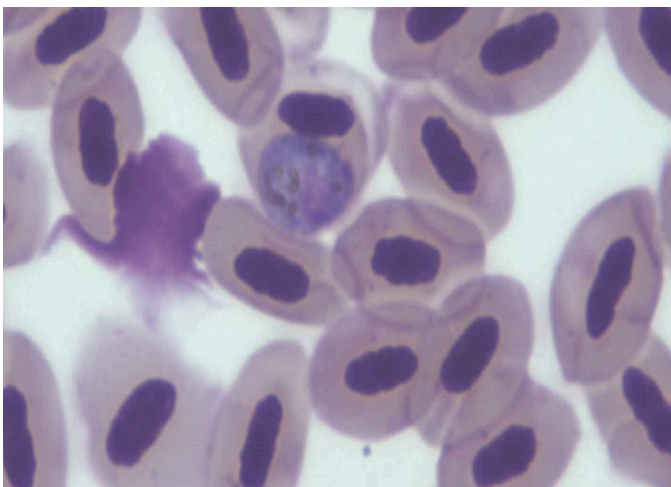
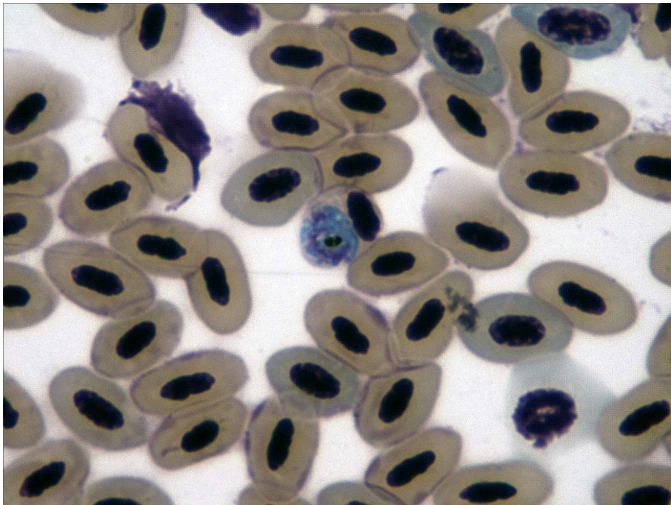


Macro

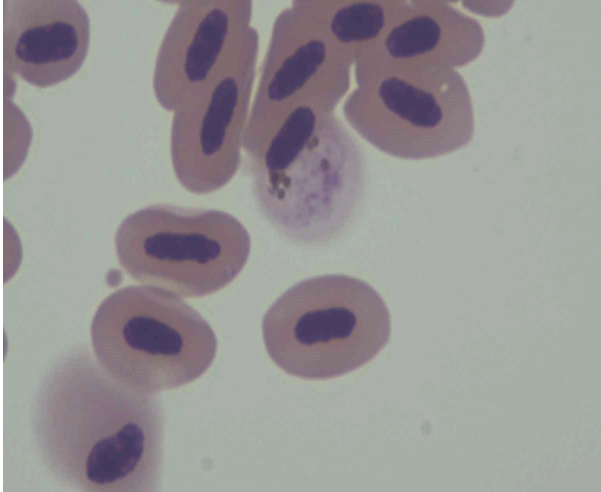
Note: *H. coatneyi* can be easily confused with *Plasmodium* parasites. A good identifier is that *H. coatneyi* will most likely be connected to the nucleus of the RBC. It is also fuller and slightly larger than the average *P. vaughni* and more oval in shape (barbell) than *P. relictum*

Plasmodium, vectored by blood-feeding mosquitoes (Diptera: Culicidae), can cause a variety of physiological damage including anemia but also the blockage of brain capillaries and the capillaries of other vital organs. We look for two species of *Plasmodium*, *P. relictum* and *P. vaughni* in addition to meronts of both of these species. Meronts are in a different life stage than the gametocytes but can be distinguished by species.

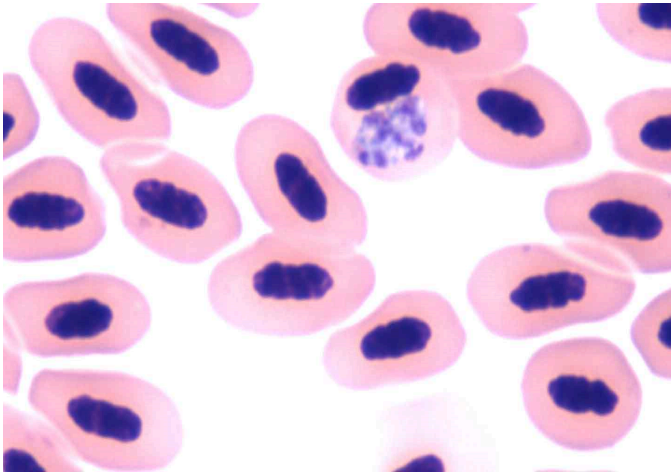
P. relictum: Characterized by an almost completely round shape, in addition it can also become detached from the cell.



Macro



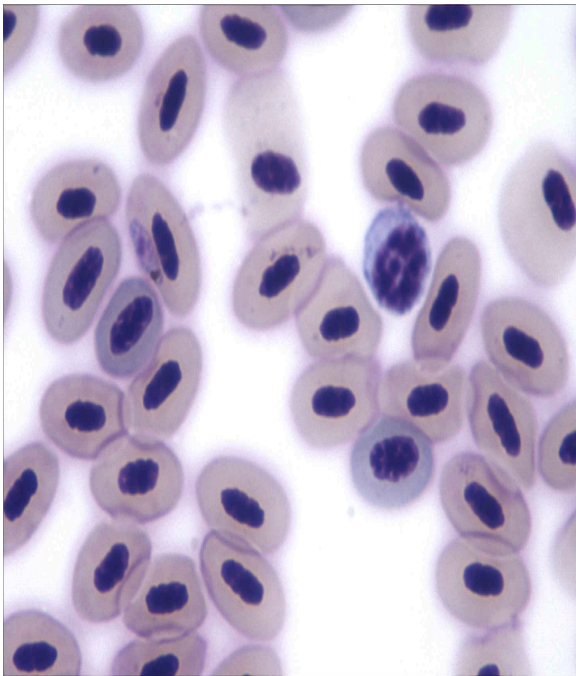
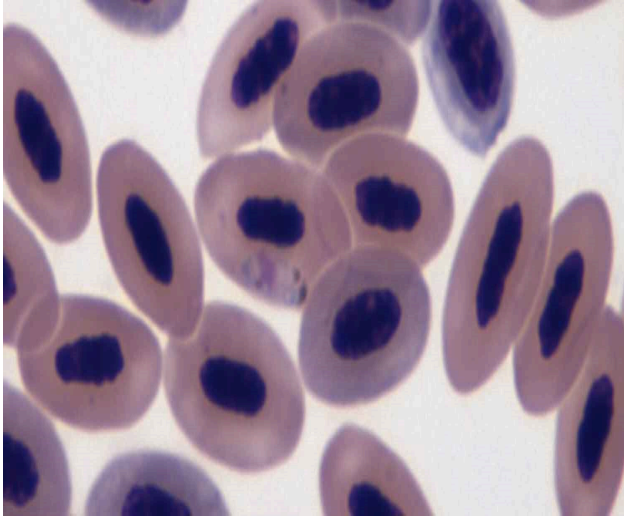
Micro



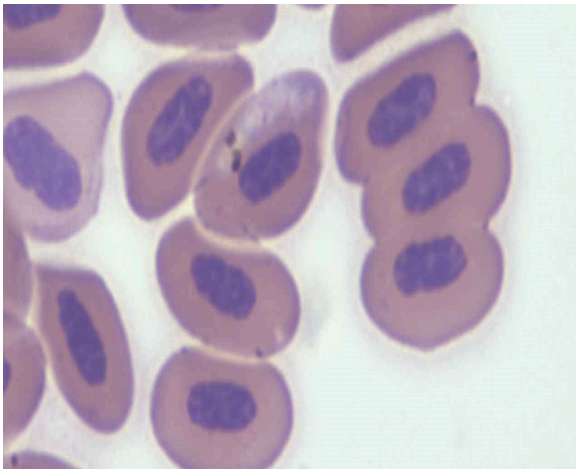
Meront

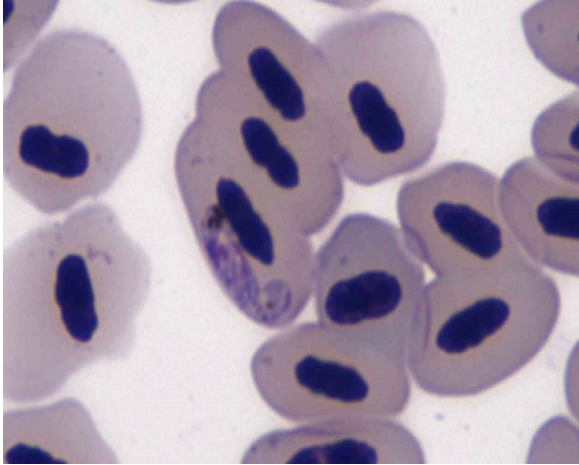
Note: Meronts of *P.relictum* are rather large and fragmented.

P. vaughni: Can look a lot like *H. coatneyi*, it is rather oval in shape and looks almost like a worm. However it is thinner than *H. coatneyi* and detached from the nucleus. *P. vaughni* is the hardest to identify particularly the meronts.

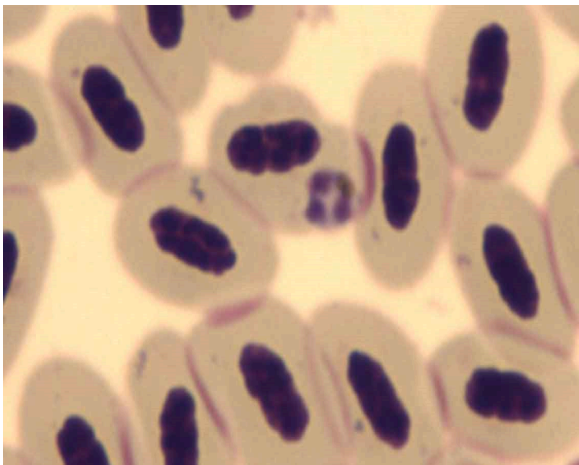
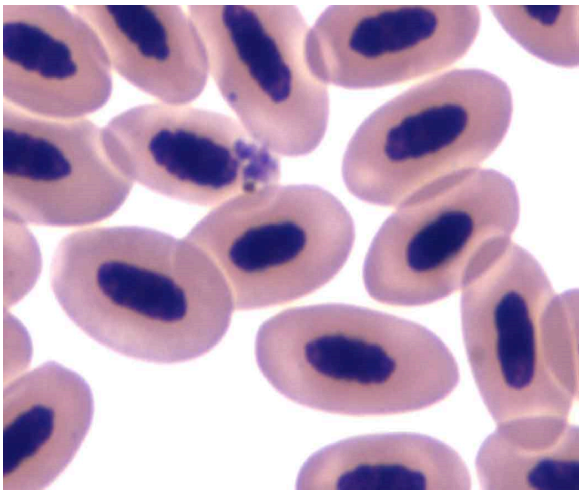


Macro



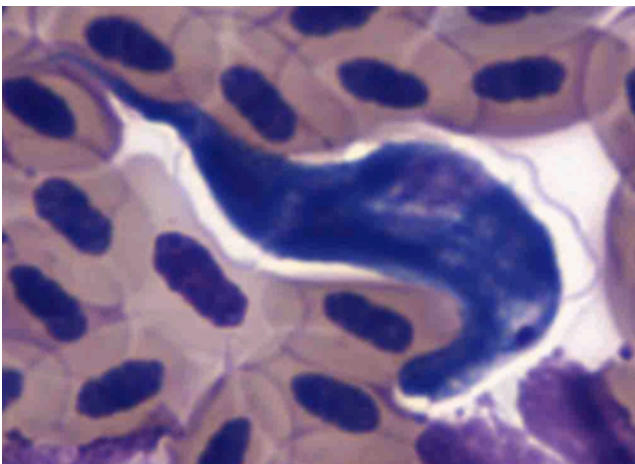


Micro

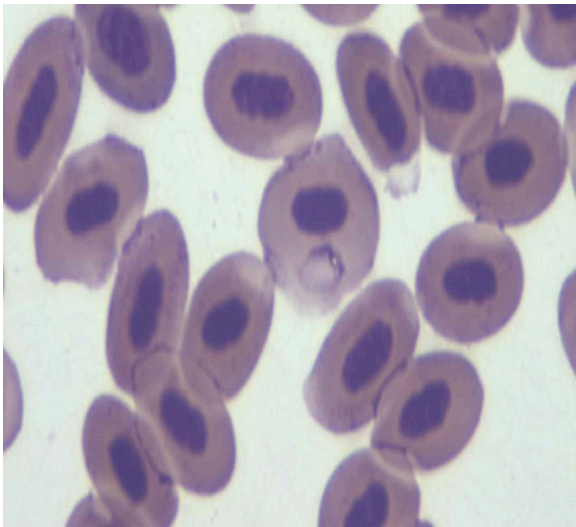


Meront

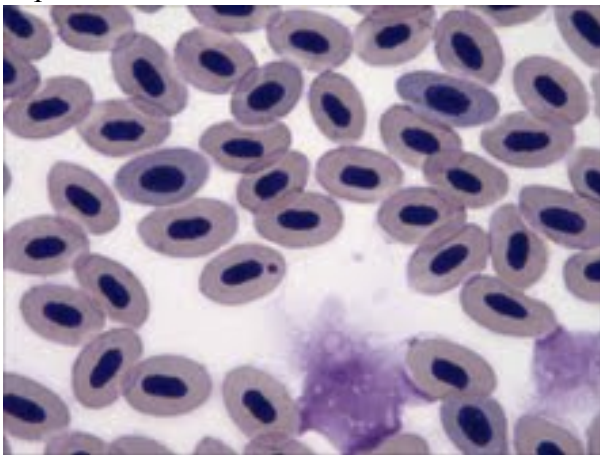
Trypanosoma avium: *T. avium* is not a malarial parasite but it is an important blood parasite and cool too!

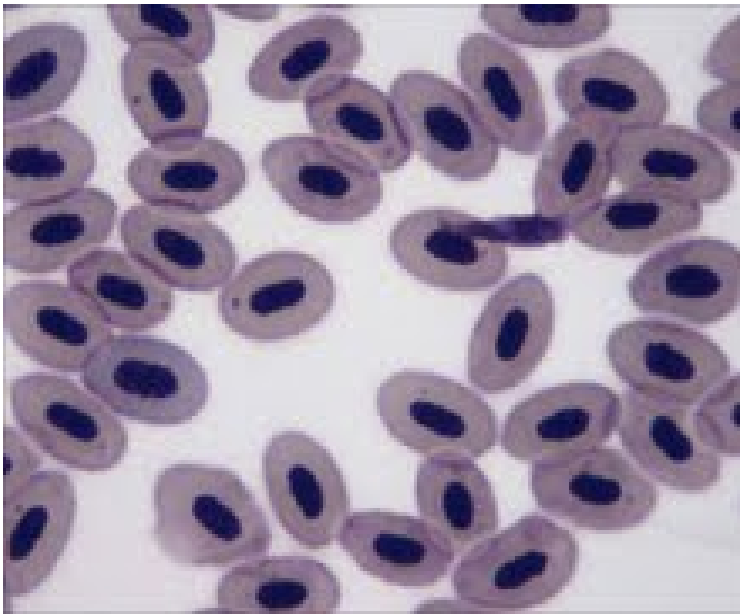
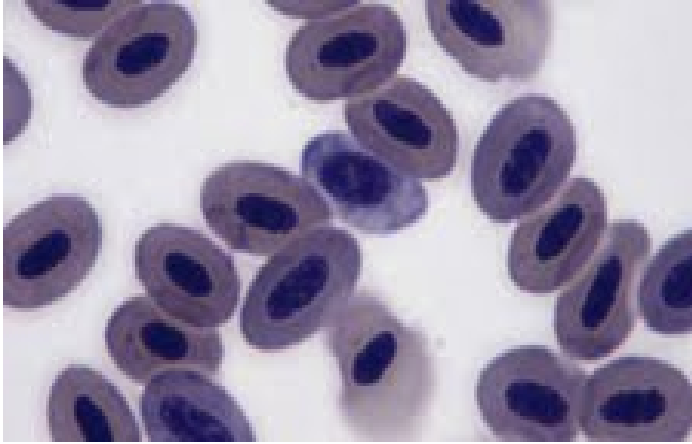


modium and Haemoproteus parasites. They are prevalent in the blood smears and are similar in shape and have a dark ring around the nucleus. Look for the ring! It is different from air bubbles.



Junk: Here are some pictures of common junk things that can be confusing as parasites or trophozoites.





*this picture is full of air bubbles that could be mistaken for trophs!

Note: For more pictures, go to the MWCS data folder on the lab Mac, then to the pictures folder. Each parasite genera has its own picture folder ☺

Score Sheets

The score sheets are pretty self explanatory. Make sure to write down both the band number and the color combination of the bird for redundancy in the band number column. In addition make sure you place the date that you scored the slide in front of the band number column, the date column is for the date the slide was made and should be on the slide.

A file with the template for the score sheets is located on the lab Mac in the Madeline folder.

How to take a Picture

The microscope closest to the computer is equipped with a Qcapture picture. To take a picture open Qcapture on the lab mac. Then focus your field on the microscope, pull the pin on the camera half way out (this will allow you to look at the field in the microscope as well as on the screen) refocus your field. Be careful, the camera is very sensitive and slight movements and will become blurry very quickly.

When ready to take a picture pull the pin out the rest of the way (allowing you to only look at the image on the computer screen. Use the software to take the picture. Name the picture with the parasite to the species level (macro or micro) and with the band number found on the slide.

Excel files:

There is a master file entitled MWCS2000sfinal with all the parasitemia counts from all the blood smears from 2000-2009.

Good Luck ☺