Sablefish (*Anoplopoma fimbria*) plasma biochemistry and hematology reference intervals including blood cell morphology

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Carla B. Schubiger[1*], M. Elena Gorman[1], Jennifer L. Johns[1], Mary R. Arkoosh[2], Joseph P. Dietrich[2]

[1]Carlson College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, USA

[2]Environmental & Fisheries Science Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Newport, Oregon, USA

*Corresponding author:

Email: Carla.schubiger@oregonstate.edu (CBS)

Abstract

Plasma biochemistry and hematology reference intervals are integral health assessment tools in all medical fields, including aquatic animal health. As sablefish (*Anoplopoma fimbria*) are becoming aquaculturally and economically more important, this manuscript provides essential reference intervals (RI) for their plasma biochemistry and hematology along with reference photomicrographs of blood cells in healthy, fasted sablefish. Blood cell counts and morphology can vary between species, precluding the use of RI's from other fish species for use in sablefish. Blood was collected for plasma biochemistry and hematology analysis between June 20 and July 18, 2019, from healthy, yearling sablefish, hatched and reared in captivity on a commercial diet. Overnight fast of 16-18 hours did not sufficiently reduce lipids in the blood, which lead to visible lipemia and frequent rupture of blood cells during analysis. Therefore, sablefish should be fasted for 24 to 36 hours before blood is collected to reduce hematology artifacts or possible reagent interference in plasma biochemistry analysis. RI's may also be influenced by feed, demographics, and production systems, warranting more studies on this topic. In sablefish, lymphocytes are the dominant leukocytes (98%), while eosinophils are rare, and basophils were not detected. Neutrophils are very large cells with Döhle bodies. These bodies are usually signs of toxic changes, but no such association was found in these fish.

Introduction

Sablefish (*Anoplopoma fimbria*), colloquially known as butterfish or black cod, are a highly valuable and fast-growing demersal species native to the continental slope of the

47 North Pacific Ocean. In 2012, U.S. harvest of wild sablefish was valued at
48 approximately $141 million (1). Wild sablefish populations have historically declined in
49 the late 1900s due to massive harvest by international fishing fleets, and as a result, the
50 catch is highly regulated to allow for currently stable wild populations (1–4). National
51 and international market demands are projected to continuously increase while harvest
52 of wild sablefish will likely remain limited, resulting in increased interest in an
53 aquaculture industry to meet sablefish demand.
54 Several research efforts and commercial projects are underway to establish sablefish
55 brood-stock, larval rearing techniques, feeds, as well as the development of net-pen
56 and landlocked recirculating aquaculture systems (RAS) (5–10). Investing in this
57 emerging aquaculture industry brings new opportunities to aquaculture communities
58 and a socioeconomically sensitive approach to satisfy market demands and create jobs
59 beyond what limited catch permits can achieve (11,12).
60 High stocking density within any culture system increases disease pressure and is a
61 ubiquitous challenge for producers and aquatic veterinarians in charge of maintaining
62 the health status of valuable finfish (13). Research efforts are ongoing to mitigate
63 disease pressure in these culturing systems (14,15). However, little is known about
64 normal physiological health parameters of sablefish. This knowledge gap leaves
65 professionals with insufficient tools to support the health needs of this industry.
66 This study's objective was to develop diagnostic references for normal ranges
67 (reference intervals; RI) of plasma biochemistry and hematology values and to
68 characterize blood cell morphology of cultured sablefish. Producers and aquaculture

healthcare professionals will be able to use these RI as a guideline and a diagnostic tool.

Material and methods

Mixed-sex fish were obtained from a captive brood-stock and rearing program conducted by the National Oceanic and Atmospheric Administration's (NOAA) Northwest Fisheries Science Center (NWFSC) at the Manchester Research Station (Port Orchard, WA). The fish were reared for one year in land-based tanks with artificial recirculating seawater at the NWFSC (Seattle, WA) and transferred to NOAA's Newport Research Station (Newport, OR) on April 18, 2019. At the start of holding, the fish had an average weight of 186 g and grew to an average weight of 440 g on the final day of sampling. In Newport, the sablefish were reared in a 2-m diameter fiberglass tank in ca. 1500 L, with flow-through seawater at ca. 10-14.5 °C. The salinity ranged from 29 to 33.9 ppt and dissolved oxygen ranged from 6.9 to 9.6 mg/L. The indoor tank was lit from above with a combination of natural (skylights) and artificial lighting. The artificial lighting was on a daily timer, providing 9 hours of light, turning on after sunrise, and turning off before sunset. Internal air temperatures ranged from 17.9 to 23.9 °C.
Fish were fed BioOregon's Biobrood (4 mm) diet at least twice per day five days per week, at approximately 1% of body weight. This diet consists of marine fish oils and fishmeal protein. The feed's composition, per manufacturer, includes 48% minimum protein; 20% minimum oil; 8.5% maximum moisture; 1.0% maximum fiber; 11% maximum ash; 18.2 MJ/kg digestible energy; and 60 ppm astaxanthin (a carotenoid).
The sablefish were visually inspected daily Monday through Friday. There were no

92 mortalities or signs of illness observed in the sablefish throughout the duration of the
93 study.
94

95 Preparation of reference animals, sample collection and
96 handling

97 Pre-analytical procedures were consistent with methodologies applicable to health
98 assessments in aquatic medicine (16). In brief, fish were fasted for 24-36 hours prior to
99 terminal blood collection to avoid lipemia. Two fish were netted and transferred to 19-L
100 buckets containing 500 mg/L tricaine methanesulfonate (MS-222) for euthanasia. After
101 the movement of the operculum stopped, fish were weighed and measured and placed
102 in dorsoventral orientation. Blood was sampled within 3 minutes of euthanasia from the
103 caudal vein. 25 gauge (25 G X 5/8), heparinized needles were inserted at the midline
104 approximately 1-2 cm caudally of the anal fin and advanced craniodorsally at an
105 approximately 60-degree angle to just ventral of the spine. Approximately two to four
106 mLs of blood per fish were collected in the heparinized syringes. Immediately after
107 collection, blood smears were prepared, and non-heparinized microhematocrit capillary
108 tubes (Fisher Scientific) were filled to determine hematocrit and total plasma protein.
109

110 An aliquot of heparinized blood (1.5 mL) was centrifuged at 1200 x g at 14 °C for 10
111 minutes. Plasma was harvested and transferred to a sterile 2 mL empty tube.
112 Heparinized whole blood and plasma samples from each fish were stored in a chilled
113 container and transported within 2-4 hours to Oregon State University Veterinary
114 Diagnostic Laboratory in Corvallis, Oregon.

115 Blood was collected from a total of 87 sablefish. However, data from 6 fish (sampled on
116 June 20 and July 2, 2019) were excluded from analysis due to grossly high lipid content
117 in the blood that could potentially cause aberrant biochemical and hematological
118 analyses and skewing statistical results. Therefore, reference intervals were ultimately
119 determined from a total of 81 sablefish of the same lineage, age, and environmental
120 conditions. Fish were collected on June 24 (2 fish), 25 (3 fish), 26 (3 fish), and July 5 (1
121 fish), 10 (32 fish), and 17 (40 fish), 2019. This study was permitted by the animal care
122 and use committee at Oregon State University (ACUP 5154).
123

124 Hematology and Biochemistry Methods

125 Heparinized whole blood and plasma samples were immediately processed upon
126 receipt by the Veterinary Diagnostic Laboratory at the Oregon State University Carlson
127 College of Veterinary Medicine in Corvallis, Oregon. The lab evaluated the biochemical
128 and hematologic parameters.
129 Plasma biochemical profiles were selected based upon prior studies in other fish
130 species (17–19). Biochemical analysis was performed via the Beckman Coulter AU480
131 automated chemistry analyzer (Beckman Coulter Inc., Brea, CA, USA) and reported in
132 conventional units. The conventional units were then converted to the International
133 System of Units (SI units) (20). The biochemical parameters analyzed are listed in Table
134 1.
135

136 **Table 1. Plasma biochemistry parameters.**

Plasma Biochemistry	Description

Parameter & Unit of Measurement	
Albumin (Alb)	Protein synthesized by the liver, important for oncotic pressure in blood and carrier function of hormones, enzymes, vitamins, etc.
Globulins (Glob)	Proteins synthesized in liver and immune system, important in immune response, liver function, blood clotting, etc.
Total Protein (TP)	Albumin and Globulin combined
Glucose	Simple sugar important energy source
Cholesterol	Lipid synthesized in cells, serves as component of cell membranes and precursor of steroid hormones and vitamin D.
Creatinine	Breakdown product from muscle metabolism, excreted by kidneys
Urea nitrogen (BUN)	Waste product produced in liver from protein digestion, excreted by kidneys
Total Bilirubin	Breakdown product of red blood cells, excreted via liver, kidneys, and gastrointestinal tract
Alanine transaminase (ALT)	Formerly called serum glutamate-pyruvate transaminase (SGPT), enzyme present in normal liver and muscle cells
Aspartate Aminotransferase (AST)	Transport enzyme in amino acid metabolism, biomarker for liver health, also known as serum glutamic oxaloacetic transaminase (SGOT)
Creatine Kinase (CK)	Enzyme marker for muscle health
Sodium (Na)	Electrolyte to maintain fluid levels and pH
Potassium (K)	Electrolyte involved in muscle and nerve activity, fluid levels and more
Chloride (Cl)	Electrolyte involved in blood volume and pressure, and pH
Calcium (Ca)	Electrolyte stored in bones, if levels increase indicative of bone, kidney, or thyroid disease and others

Phosphorus (P)	Mineral in bones and teeth, involved in nerve signaling and muscle contractions, abnormal levels indicative of kidney, liver, or bone diseases
Total CO_2	Blood-gas waste product, part of bicarbonate involved in pH level
Anion Gap	Related to electrolyte panel, indicates pH levels

137

138

139 Measurement of all hematologic parameters was performed manually within 24 hours of
140 receipt. Heparinized whole blood was utilized to assess packed cell volume (PCV), total
141 protein (TP), and manual cell counting. PCV was obtained by the collection of whole
142 blood into plain hematocrit tubes and measurement post-5-minute centrifugation. Total
143 plasma protein was evaluated by a refractometer (Reichert, Depew, NY, USA).
144 Manual cell counts were performed by two board-certified clinical pathologists (EG and
145 JJ). Leukocyte and thrombocytes were stained by transferring 20 µL whole blood into
146 1.98 mL Natt-Herrick Solution (Vetlab supply, Palmetto Bay, FL, USA) for a 1:100
147 dilution. The mixture was allowed to incubate for 20-30 minutes at room temperature
148 then counted via Neubauer hemocytometer (Bright-Line, Hausser Scientific, Horsham,
149 PA) using a standard protocol (17,21,22). Leukocytes were counted in nine primary
150 squares, and the total was divided by nine and multiplied by 100 (dilution factor).
151 Thrombocyte counts were obtained by counting thrombocytes in the center main-square
152 of the hemocytometer then multiplying by 1000.
153 Blood smears were submitted on glass slides and stained with Modified Wright's stain
154 (Thermo Fisher Scientific) via an automated method (HemaTek 2000, Siemens
155 Healthcare, Erlangen, Germany). Leukocytes were identified based upon characteristics

156 described for other related marine species (23,24), and morphologic identity was initially
157 agreed upon by both pathologists (EG and JJ). Leukocyte differential counts were
158 performed by an individual pathologist (EG) to minimize inter-observer variability.
159

160 Reference interval calculations

161 Blood values were collected into Excel spreadsheets and reference intervals calculated
162 with the macro add-in Reference Value Advisor V 2.1 software (25). The software
163 follows recommendations and guidelines by the Clinical and Laboratory Standards
164 Institute (CLSI), the National Committee for Clinical Laboratory Standards (NCCLS),
165 and the International Federation of Clinical Chemistry (IFCC). The software determined
166 that the sample size was large enough to compute nonparametric reference intervals
167 and also determined the 90% confidence intervals of the limits of the nonparametric
168 reference intervals by a bootstrap method (25,26).
169

170 Results

171 Plasma biochemistry reference intervals

172 The reference intervals for measured plasma biochemistry parameters in the 81
173 sufficiently fasted sablefish is presented in Table 2. Overnight fasting of 16-18 hours
174 was not sufficient to reduce visible lipemia of the blood in three of the sablefish sampled
175 on June 20, 2019 (animals #32, #33, #34). Also, an unscheduled blood collection was
176 performed on July 2, 2019, with fish that were fasted for only 16-18 hours leading again
177 to grossly lipemic samples (animals #47, #48, #49). Consequently, the protocol was
178 adjusted to increase the fast time to 24-36 hours. Therefore, six of the 87 animals were

179 excluded from the reference interval calculations due to the severely lipemic condition of

180 the plasma samples. This condition displayed as visible turbidity in some samples (S1

181 Fig) and severe blood cell rupture in others.

182

183 **Table 2. Reference intervals for plasma biochemistry parameters of fasted**

184 **sablefish.**

Parameter; n = 81	SI units	Conventional units
Albumin (Alb)	6.1 – 13 g/L	0.61 – 1.3 g/dL
Globulin (Glob)	19.1 – 31.9 g/L	1.91 – 3.19 g/dL
Total Protein (TP)	25.2 – 44.9 g/L	2.52 – 4.49 g/dL
Glucose	0.95 – 4.91 mmol/L	17.1 – 88.4 mg/dL
Cholesterol	6.34 – 12.48 mmol/L	245.2 – 482.5 mg/dL
Creatinine	0 – 140.46 µmol/L	0 – 1.59 mg/dL
Urea nitrogen (BUN)	0.07 – 0.14 mmol/L	2.0 – 4.0 mg/dL
Total Bilirubin	1.71 – 3.42 µmol/L	0.1 – 0.20 mg/dL
Alanine aminotransferase (ALT, SGPT)	0 – 17.8 U/L	0 – 17.8 U/L
Aspartate aminotransferase (AST, SGOT)	0 – 1.88 µKat/L	0 – 110.4 U/L
Creatine kinase (CK)	0.09 – 1.49 µKat/L	5.1 – 87.4 U/L
Sodium (Na)	168.0 – 179.9 mmol/L	168.0 – 179.9 mEq/L
Potassium (K)	1.2 – 3.86 mmol/L	1.2 – 3.86 mEq/L
Chloride (Cl)	142.0 – 153.0 mmol/L	142.0 – 153.0 mEq/L
Calcium (Ca)	2.63 – 3.35 mmol/L	10.51 – 13.38 mg/dL
Phosphorus (P)	4.17 – 8.41 mmol/L	12.92 – 26.06 mg/dL
Total CO_2	4.64 – 8.70 mmol/L	4.64 – 8.70 mEq/L
Anion Gap	18.1 – 27.0 mmol/L	18.1 –27.0 mEq/L

185 Table 2 legend: Table 2 lists reference intervals for plasma biochemistry parameters of

186 sablefish that were fasted 36 to 48 hours. Data were collected in conventional units and

187 converted into SI units according to (20).

188

Hematology Reference Intervals

Hematologic parameters, including packed cell volume (PCV), plasma protein (PP), and cell counts, were collected manually from the 72 fish sampled on July 10 and 17, 2019. Thrombocyte counts were only available from 60 animals due to early technical difficulties with staining. All data are presented in Table 3.

Table 3. Cell blood counts (CBC) were available from 72 animals, with the exception that thrombocyte counts were only available from 60 animals.

Parameter	n = 72 (*n = 60)
Packed Cell Volume (PCV)	35.7 – 61.8 %
Plasma Protein (PP)	4.3 – 7.92 g/dL
Thrombocytes *	92,100 – 357,475 /µL
White Blood Cell (WBC)	4,333 – 33,083 /µL
Neutrophils	1.8 – 23.4 /µL
Lymphocytes	66 – 96 /µL
Monocytes	0 – 12.1 /µL
Eosinophils	0 - 2.2 /µL
Basophils	0 /µL

Table 3 legend: PCV, PP, and blood cell counts were manually performed from fish sampled on July 10 and 17, 2019. Thrombocyte counts were manually performed from 60 fish sampled on July 10 and 17, 2019. Data present reference intervals.

Morphology of blood cells was similar to those noted in other fish species (23,24,27). Erythrocytes are oval with a round to ovoid nuclei (Fig 1a,b,c). Polychromatic erythrocytes were commonly found in sablefish, which indicates an active turnover of

206 erythrocytes (Fig 1a). Additionally, fish have a thin barrier between hematopoietic tissue
207 and the vasculature; hence, immature erythroid cells were common and may comprise
208 more than 10% of all erythrocytes in circulation (23,27) (Fig 1c). Immature erythrocytes
209 are smaller and more round than mature erythrocytes with basophilic cytoplasm (Fig
210 1a). Mitotic figures were not readily identified in blood smears of this population.
211
212 **Fig 1a. Lymphocytes: Granular lymphocytes.** Granular lymphocyte (GL), surrounded
213 by rounder, basophilic immature erythrocytes (IE) that are smaller than the more mature
214 erythrocytes (EC). Overall erythrocytes are in various stages of maturity contributing to
215 a distinct polychromatic appearance, ranging from slightly eosinophilic to basophilic
216 (Wright's stain x100 objective).
217
218 **Fig 1b. Lymphocytes: Mature lymphocyte and monocyte.** Mature lymphocyte (L)
219 and monocyte (M) with typical large oval eccentrically-positioned nucleus and a
220 moderate amount of basophilic cytoplasm that contains three discrete cytoplasmic
221 vacuoles. Mature lymphocytes are characterizes as round cells with large, round
222 nucleus with condensed chromatin and scant basophilic cytoplasm. Lymphocytes often
223 display blebbing of the cell borders (Wright's stain x100 objective).
224
225 **Fig 1c. Lymphocytes: Reactive lymphocyte.** Reactive lymphocyte (with Golgi body
226 left to the nucleus, RL), small lymphocyte (L) with blebbing and neutrophil (N). Reactive
227 lymphocytes are large cells, with a more open chromatin pattern and increased
228 amounts of basophilic cytoplasm (Wright's stain x100 objective).

229

230 In sablefish, lymphocytes predominated and comprised up to 98% of the total
231 leukocytes present in healthy individuals. Lymphocyte morphology was similar to that of
232 other species, and lymphocytes were identified as round cells with a high nucleus to
233 cytoplasm ratios (N:C ratios), round nuclei and condensed chromatin (Fig 1a-c). Cell
234 borders of lymphocytes were frequently blebbed (bulging plasma membrane) (Fig 1c).
235 Reactive lymphocytes were also similar to those in other species, with cells being larger
236 than mature lymphocytes, more open chromatin patterns, and increased basophilic
237 cytoplasm. Occasional Golgi bodies were noted (Fig 1c). Additionally, granular
238 lymphocytes were occasionally found (Fig 1a). Neutrophils were one of the largest
239 leukocytes identified in sablefish (Fig 1c, 2, 3). Neutrophils were oval with oval, clefted,
240 or segmented nuclei surrounded by moderate to abundant amounts of slightly
241 eosinophilic cytoplasm. Most neutrophils contained pale blue cytoplasmic inclusions
242 consistent with Döhle bodies. These bodies are often signs of toxic granulation,
243 inflammation or bacterial infection, but appeared to have no association with other toxic
244 changes in these fish (Fig 1c, 2, 3) (28–30).

245

246 **Fig 2. Neutrophils.** Neutrophils (N) are large leukocytes with large oval, clefted, or
247 segmented nuclei and moderate to large amounts of slightly eosinophilic cytoplasm that
248 often contains pale blue cytoplasmic inclusions consistent with Döhle bodies (Wright's
249 stain x100 objective).

250

251 **Fig 3. Neutrophil (N) and Monocyte (M).** Note similar size between these cells. Mature
252 erythrocytes (EC) also identified (Wright's stain x100 objective).
253
254 Monocytes were larger than lymphocytes and similar in appearance to monocytes in
255 other non-mammalian species (Fig 1b, 3, 4). Monocytes contained oval eccentrically-
256 positioned nuclei; amoeboid nuclear shapes were less frequent. Monocytes had small to
257 moderate amounts of variably basophilic cytoplasm and frequently contained discrete
258 cytoplasmic vacuoles (Fig 1b, 3, 4). Cell borders were distinct and non-blebbed, making
259 them easily distinguishable from lymphocytes. Eosinophils were rare in healthy
260 sablefish. These were small cells with a high N:C ratio (Fig 5). Nuclei of eosinophils
261 were oval and typically eccentric, and cytoplasm contained small, bright eosinophilic
262 granules (Fig 5). Basophils were not found. Thrombocytes were found individually and
263 in small aggregates (Fig 4, 6). Thrombocytes were small and typically oval to elongate
264 in shape. Few round forms were also noted. Nuclei of thrombocytes were pleomorphic,
265 ranging from round to oval to reniform (Fig 4, 6). The cytoplasm was clear with small
266 cytoplasmic granules occasionally seen.
267
268 **Fig 4. Monocytes.** Monocyte (M) contains several, variably sized, discrete cytoplasmic
269 vacuoles and distinct, smooth cell borders, in contrast to the two lymphocytes (L) that
270 display distinct blebbing. Also present is a thrombocyte (T) with a bi-lobbed nucleus
271 (Wright's stain x100 objective).
272

Fig 5. Eosinophils. Eosinophils (E) are slightly larger than mature lymphocytes (L) and have large, ovoid, eccentric nuclei and small amounts of cytoplasm that contains small, bright eosinophilic granules (Wright's stain x100 objective).

Fig 6. Thrombocytes (T). Thrombocytes are found individually and in small aggregates in well-prepared blood smears. Thrombocytes are small and typically oval to elongate and commonly displaying nuclear pleomorphism with round to oval to reniform nuclei. The cytoplasm of thrombocytes is usually clear with occasional, small cytoplasmic granules (Wright's stain x100 objective).

Discussion

This study addresses an urgent need associated with the development of a sustainable and highly profitable emerging aquaculture species. Establishing a sablefish aquaculture industry would contribute to the livelihoods of coastal (and inland) communities along the northern Pacific Ocean. Species-specific biochemistry and hematologic reference intervals (RI) derived from a healthy reference population are crucial for adequate health monitoring of any species. Particularly crucial is the health monitoring of species new to culturing. Management and feeding practices, and disease treatment regimens can be appropriately adjusted in correlation to physiological parameters of the fish determined from health monitoring. Blood cell counts are valuable tools in assessing the general health status of the fish and can alert a health care provider to infections and environmental or management issues, e.g., problems with water quality or nutrition.

296

297 An important observation was that fasting for 24-36 hours was necessary to obtain
298 blood samples with lipid content low enough to not interfere with plasma biochemistry or
299 hematology analysis. High amounts of triglycerides in human blood has been found to
300 significantly interfere with measurements of phosphorus, calcium, creatinine, and total
301 protein due to an interaction with the analyzer and reagents (31,32). Plasma protein
302 measured via a refractometer was predictably increased relative to plasma total protein
303 measured by an automated method as lipids refract light and can falsely increase
304 refractometric measurement of protein. High lipid content is also suspected of causing
305 premature lysis of blood cells (33). If fasting is not possible, lipid removal by
306 centrifugation or lipid extraction with polar solvents is possible (34,35). The Clinical and
307 Laboratory Standards Institute (CLSI) recommends ultracentrifugation, but this
308 procedure is not always a practical option (36). Alternatives that might be more practical
309 for onsite blood diagnostics are dilution of the serum sample or usage of serum blanks
310 (35). However, if blood samples are necessary for diagnostics in disease events, versus
311 routine health checks, lipemic blood samples might be less common as inappetence is
312 often the first clinical sign of problems in a culturing system. For scheduled health
313 checks, extended fasting of at least 24 hours is recommended for sablefish based on
314 the results presented in this study. For our analysis, we excluded samples with grossly
315 visible lipemia (milky, turbid appearance), because of possible interference with
316 biochemistry values (animals #32-34, sampled on June 20) and blood samples with
317 moderate cell rupture that was likely caused by lipemia (animals #47-49, sampled on
318 July 2). To present how these exclusion criteria influenced the plasma biochemistry

intervals, the complete data set of all 87 fish is presented as supplemental material (S1 Appendix). The exclusion of these insufficiently fasted fish (16-18 hours) notably widened the glucose range, but decreased the upper RI limits of cholesterol, creatinine, bilirubin, and the anion gap. Changes to other parameters were smaller and often in the decimals. A comparison of all values is presented as supplemental material (S1 Appendix).

The fish used in this study received a commercial diet at 1% of their body weight distributed over at least two feeding events per day. Recent studies recommend feeding sablefish to satiation once every other day to allow for adequate feed conversion and consistent intake of the feed (37,38). Reduced feed intake has been observed after four days of daily feeding, attributed to a prolonged gastrointestinal passage in this species (37) (and personal communication with Ron Johnson). Likely that the delayed clearance of blood lipids, experienced in this study, is a reflection of that observation.

A curious question is whether the observed affinity for high blood lipid concentration reflects the general tender consistency of the meat in this species, which gave rise to the colloquial name “butterfish”. However, the energy density of sablefish is relatively low compared with other pelagic fish from the North Pacific, and only low to moderate amounts of the wet mass are attributed to lipids (39). A muscle lipid content of up to 14% has been reported in sablefish with higher amounts of lipids stored in the bones (39–43). Nevertheless, sablefish seem to have a well-adapted fat metabolism. Earlier studies have shown that prolonged starvation of five months in a laboratory setting did not result in significant protein catabolism. Instead, fish survived by utilizing their fat

342 storage over muscle tissue, making sablefish generally well adapted to infrequent meals
343 in the wild (41,44). Sablefish are identified as opportunistic feeders, whose food sources
344 can alter by location and age (45). Older and spawning fish commonly reside in deeper
345 water, while the larvae hatch in the deep and then migrate up, and younger fish are
346 more commonly found in shallower waters (10,45,46). Besides the ability to travel very
347 long distances (an average of 191 km per year (47)), sablefish can also undergo an
348 impressive diel vertical migration in pursuit of some of their vertically migrating prey by
349 ascending to the surface at night and descending to waters of 750 m or greater before
350 sunlight (45). This exposes the animal to stark differences in temperature and pressure.
351 More detailed reasons for these migrations and the intricacy of sablefish lipid physiology
352 are still under further investigation.
353 Animal husbandry and management can have a substantial impact on blood reference
354 ranges. In a study of blood RIs in sunshine bass, the only biochemical reference
355 intervals that were similar in three different holding systems were glucose and cortisol
356 (48). Similarly, previous studies in sablefish describe that tank design and water
357 temperature can influence survival, anatomical deformities, meat quality, and ovarian
358 development (5–7). Moreover, studies found that blood values in sturgeon can differ
359 among different hatcheries (19). Stress is reported to alter the fish's ability for
360 osmoregulation, which will affect electrolyte concentrations in the blood, often resulting
361 in chloride loss and high glucose values (48,49). Fish sampled in this study had a wide
362 range of glucose levels (0.95–4.91 mmol/L, 17.1-88.4 mg/dL). This was mostly driven
363 by noticeably higher glucose levels in the last ten of 32 fish and 40 fish sampled on July
364 10 and July 17, respectively (S1 Appendix). Most of the glucose measurements were in

365 the upper teens to mid-forties mg/dl (approximately 1-2.5 mmol/L). The later upward
366 trend of glucose levels may be due to the repeated netting of fish from the holding tank
367 during the two to three hours of sampling. Chloride (142-153 mmol/L, 142-153 mEq/L)
368 was below what has been suggested as normal for marine teleosts (165-175 mmol/L,
369 165-175 mEq/L), but slightly higher than reported in Atlantic salmon (125-143 mmol/L,
370 125-143 mEq/L) which were regarded relatively stress-resistant, and much higher than
371 reported in Atlantic sturgeon (116-131 mmol/L, 116-131 mEq/L) (19,50,51). These
372 sources would also suggest that the sablefish RI's of monovalent sodium (168-179.9
373 mmol/L, 168-179.9 mEq/L) and potassium (1.2-3.86 mmol/L, 1.2-3.86 mEq/L), and
374 bivalent calcium (2.63–3.35 mmol/L, 10.51-13.38 mg/dL) are lower than in other marine
375 teleosts (175-200 mmol/L, 5-10 mmol/L and 3-5 mmol/L, respectively) (19,50). The
376 divergence of these parameters with reference values in older literature suggests that
377 further studies of fish species are warranted that take into account the demographics,
378 feeds, and production systems. Sex can also affect blood reference intervals (50). All-
379 female stocks are preferred in the production of many food fish species, including
380 sablefish because female fish grow larger than males (6,52). All-female stocks can be
381 achieved by mating females with neomales, which are genetic females who express
382 phenotypically male. This can either be achieved by the oral administration of
383 testosterone or by temperature management during the sexually undifferentiated phase
384 early in development (6). The mixed-sex population sampled in the present work
385 warrants further comparison and investigations into the physiology of this species. In
386 addition, the present sablefish blood RI should be compared to blood values of sablefish
387 that are cultured in other production systems like recirculating aquaculture systems

(RAS) and marine net pens, in different age groups, and from progeny that comes from cultured rather than wild, captive brood-stock.

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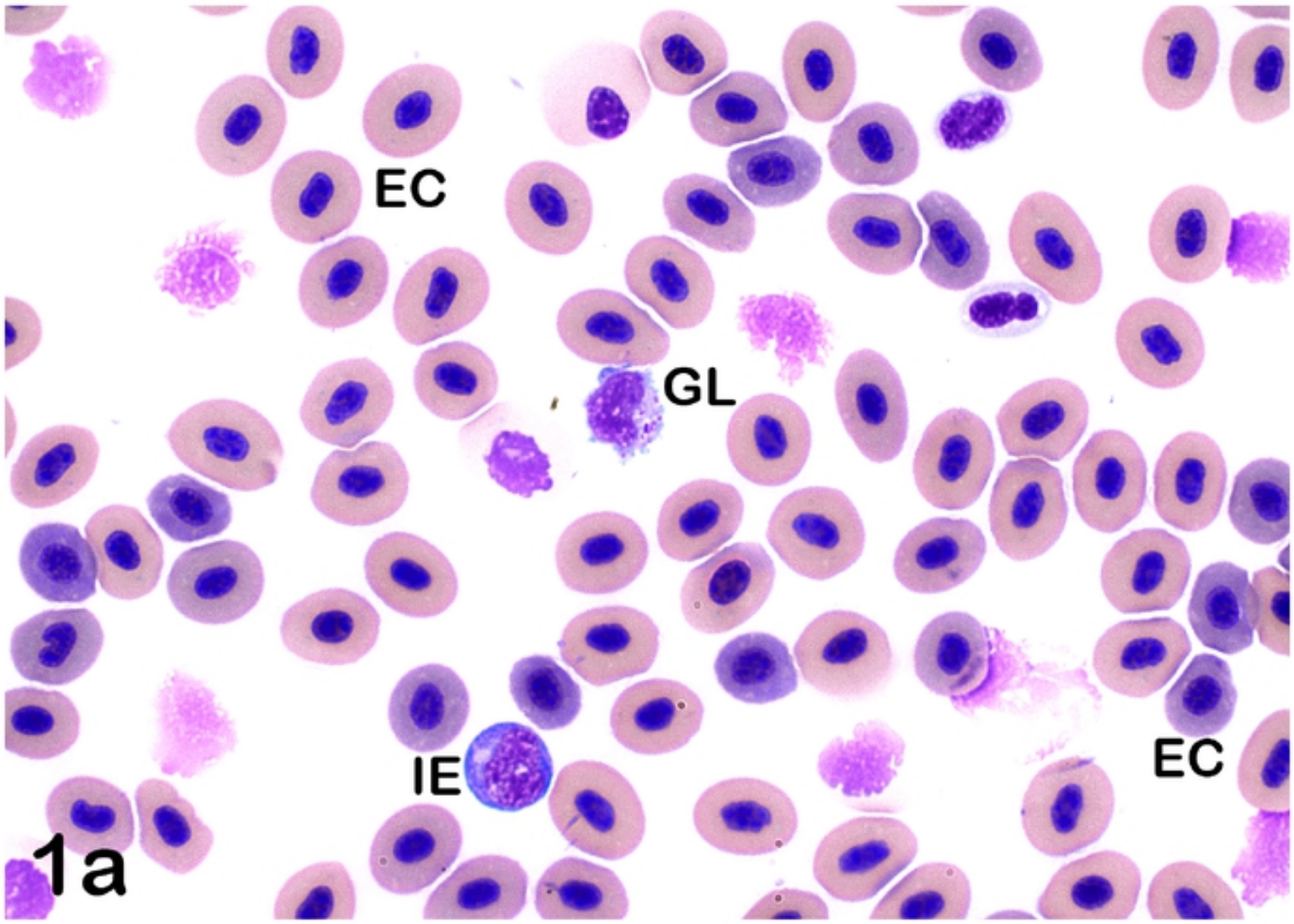
Supporting information

S1 Fig. Lipemic blood. Photograph of lipemic blood sample from fish #34 on June 20th, 2019, after centrifugation. A packed cell fraction (bottom of tube) is overlaid by a turbid, pale-beige plasma. The turbidity indicates a significant amount of blood lipids.

S1 Appendix. Raw data: Plasma biochemistry reference intervals. Data set includes lipemic samples, RI calculation reports for each biochemistry parameter, and a comparison of the RI of 87 fish (with lipemic samples) versus 81 fish (without lipemic samples), plus all conversions from the conventional units to the SI units.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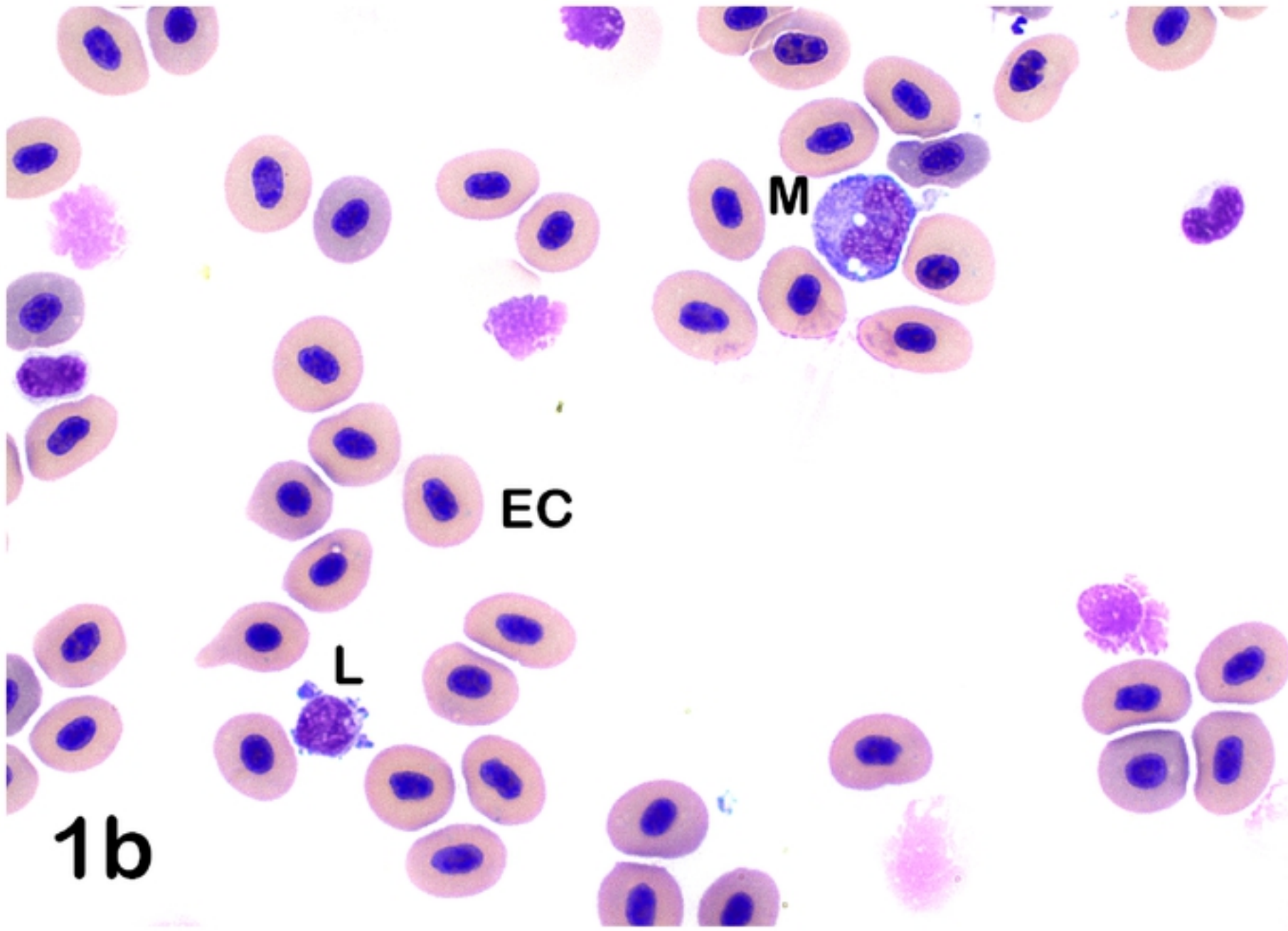
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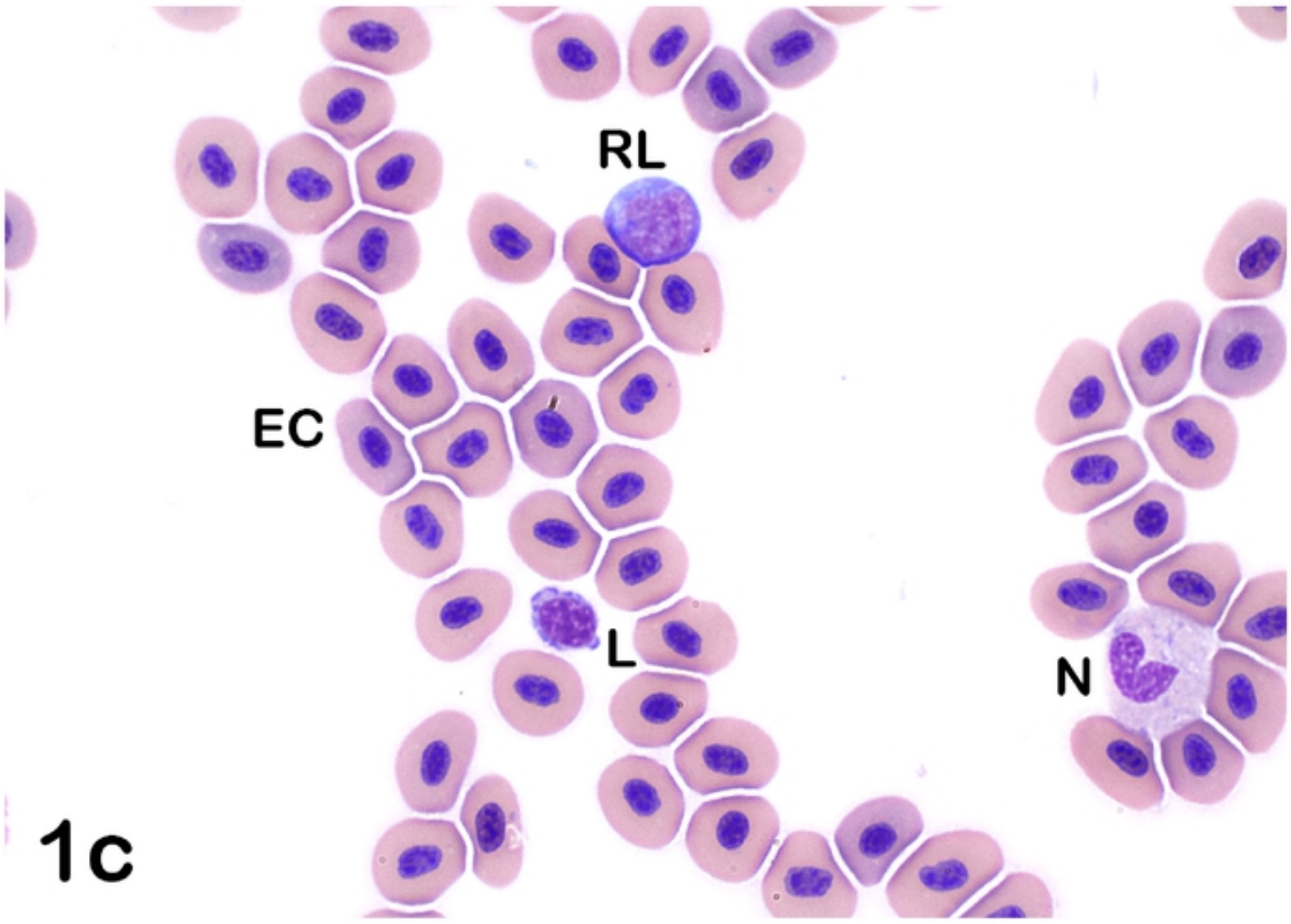

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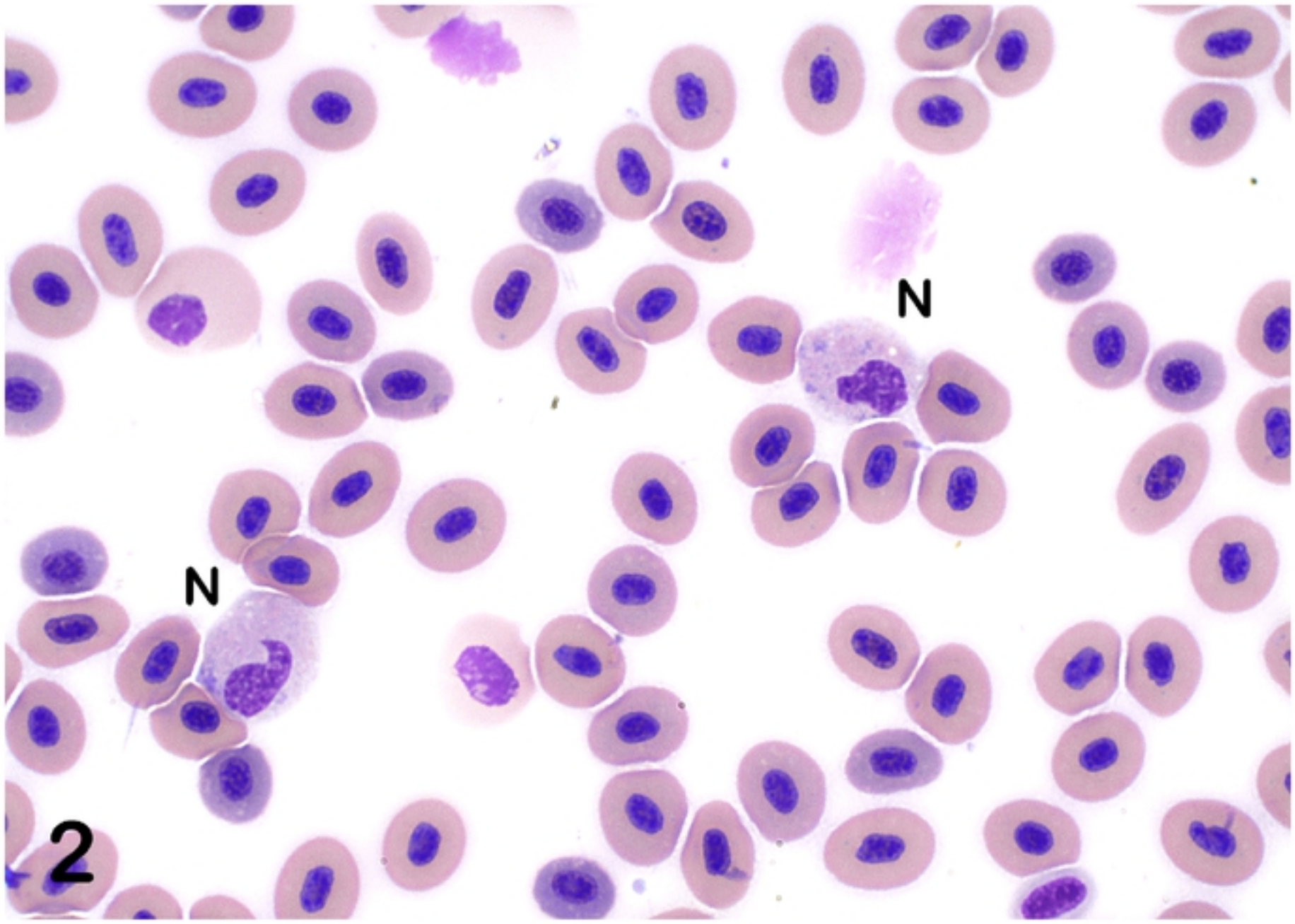
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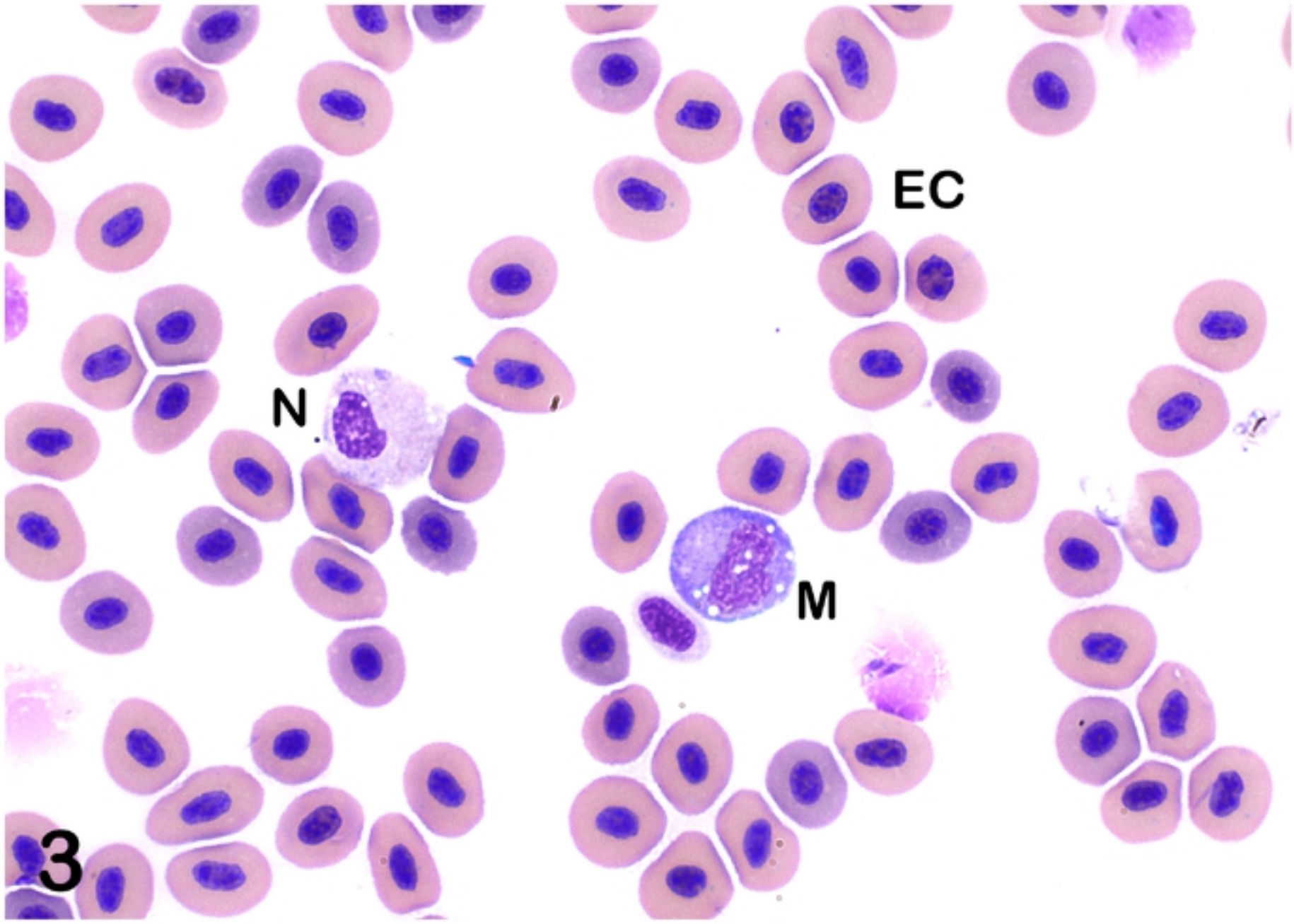
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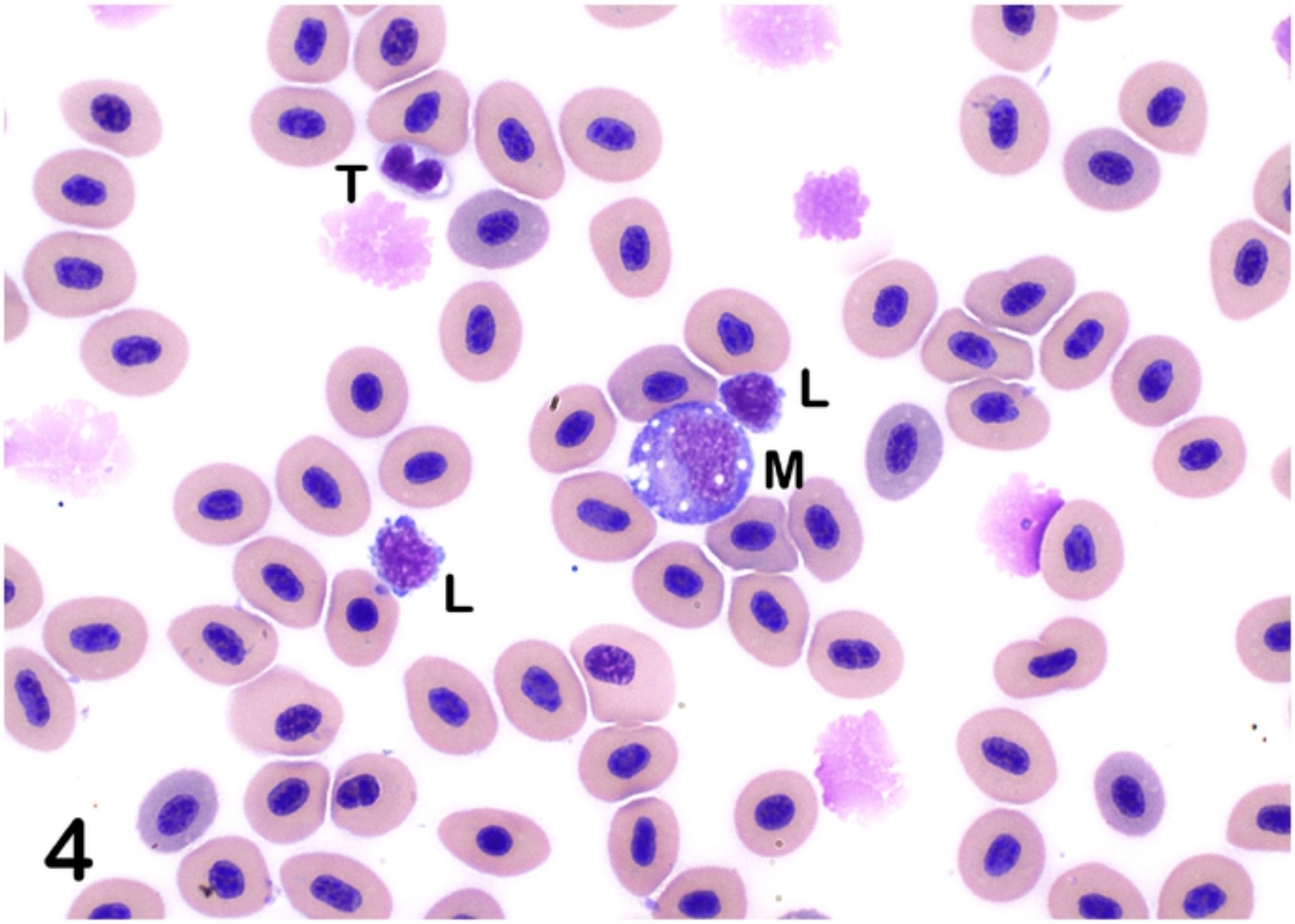
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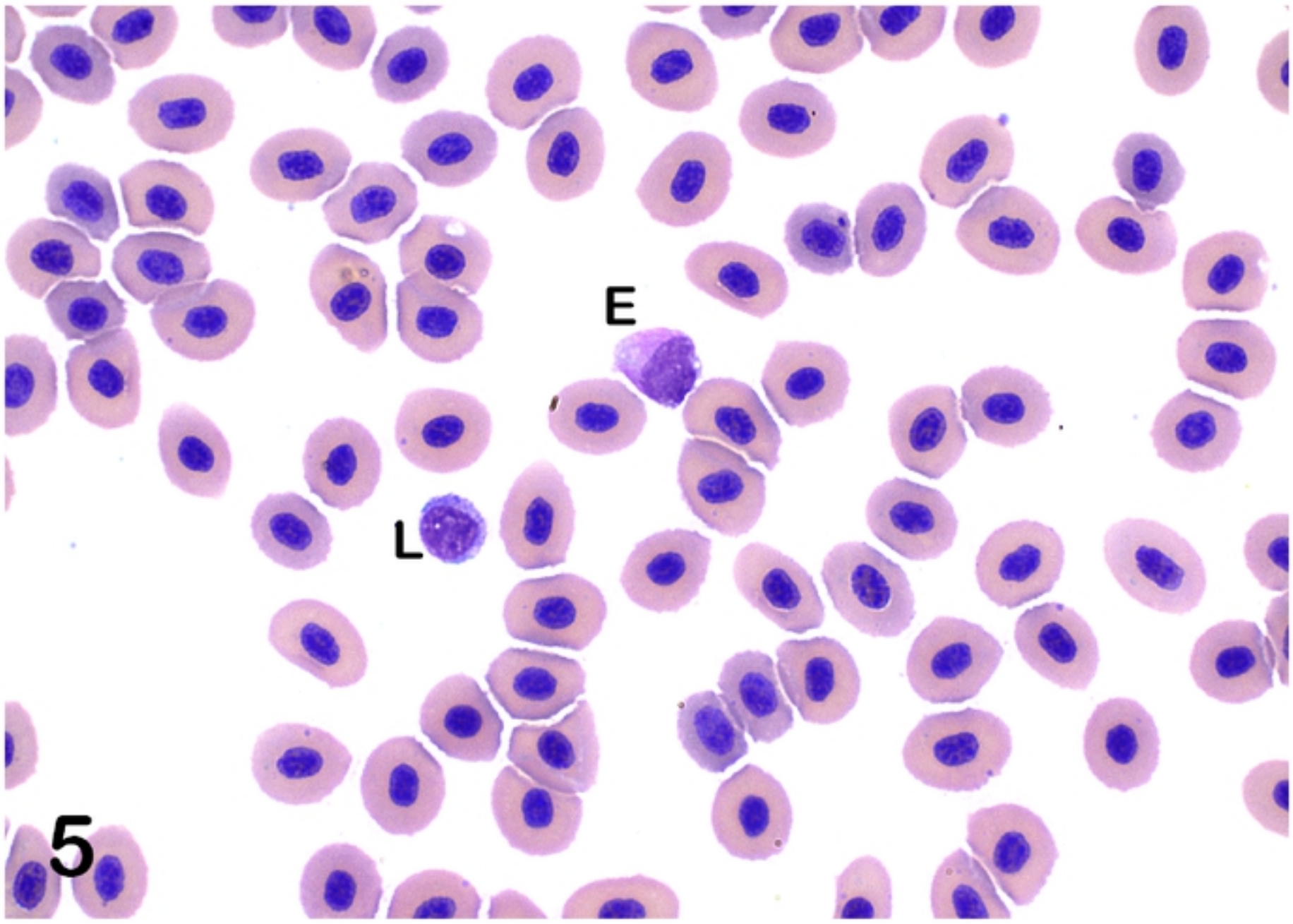
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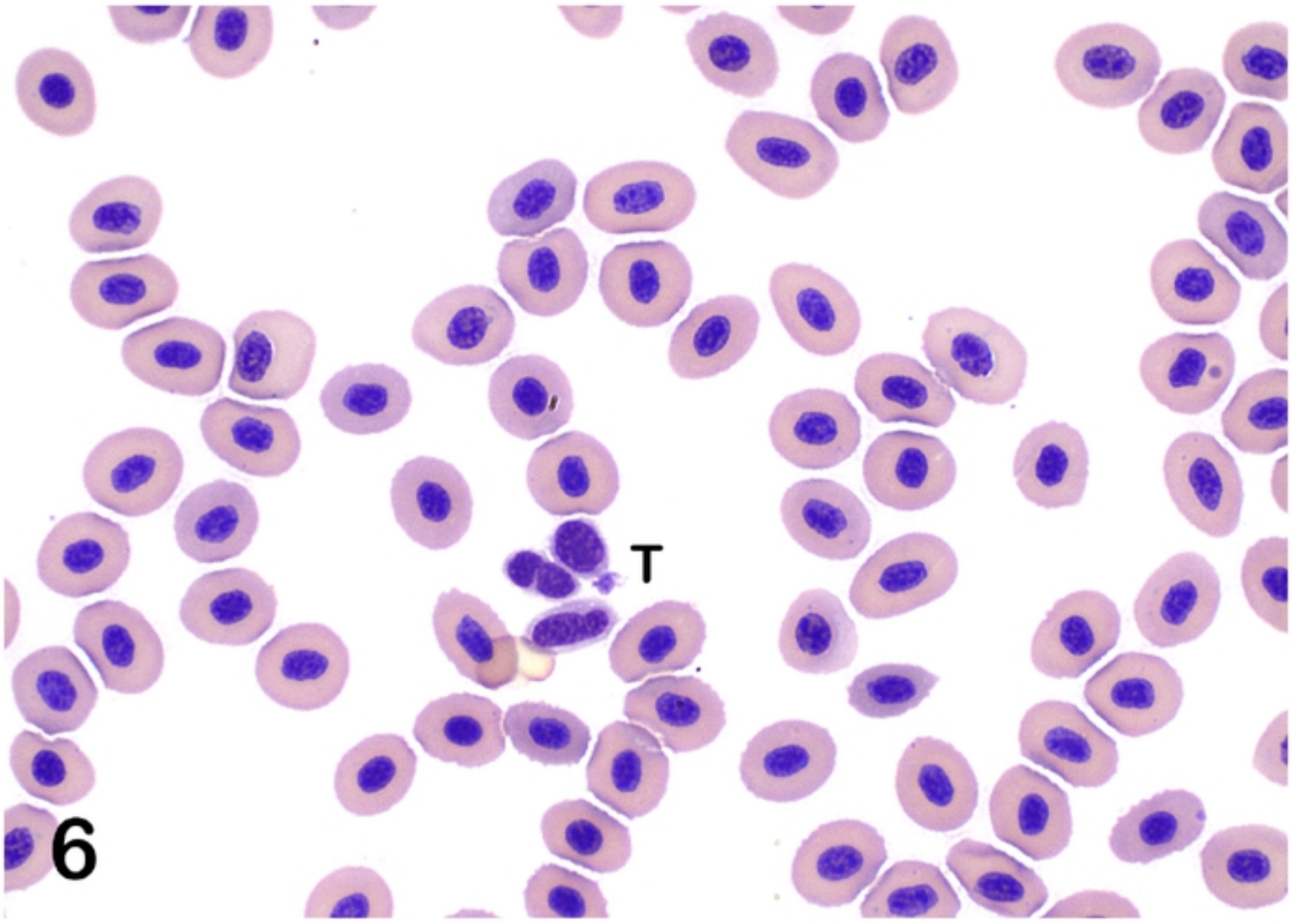
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