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ABSTRACT. – In 2015, nearly 4000 critically endangered Palawan forest turtles (*Siebenrockiella leytensis*) were confiscated on their native island of Palawan in the Philippines after being illegally harvested for the international wildlife trade. Local conservation biologists and an international team of veterinary and husbandry personnel evaluated, treated, and repatriated the majority of turtles (88%) over a 3-mo period. Common pathologic findings included ophthalmic, dermatologic, musculoskeletal, and gastrointestinal lesions, including keratitis, osteomyelitis of the shell and digits, pododermatitis, and colonic nematodiasis. Hemogram results indicated severe leukocytosis in many individuals. Specimens for genetic analysis and molecular diagnostics were archived, and several intact carcasses were established as museum specimens. International collaboration may be required to ensure the confiscation and survival of illegally traded endangered wildlife, with ongoing efforts toward enhancing the law enforcement, husbandry, and veterinary capacity of range country personnel.

KEY WORDS. – Palawan forest turtle; *Siebenrockiella leytensis*; confiscation; poaching; veterinary

The global illicit trade in wildlife and wildlife parts is well organized and lucrative, ranking only behind the trade in illegal weapons, drugs, and human trafficking in annual revenue (Jacobson and Daurora 2014). Wildlife trafficking is intertwined with other illegal activities (Jacobson and Daurora 2014) and can cause extinction of species (Brook et al. 2014). Although much of the illegal wildlife trade involves high-profile species such as elephants (Elephantidae) and rhinos (Rhinocerotidae), trade in less publicized species, such as turtles (Testudines), is also widespread and profitable (Stanford et al. 2020; Sy et al. 2020). Consumption of turtles by humans caused the extinction of several species over the past 500 yrs, and it remains the primary extinction risk for many of the world's most endangered turtle species currently (Stanford et al. 2020).

The Palawan forest turtle (*Siebenrockiella leytensis*) was described as *Heosemys leytensis* by Taylor in 1920. Its locality was reported to be Leyte in the Philippines, but it remained poorly known and rarely seen for > 80 yrs. By the early 21st century, the species was considered to be critically endangered (International Union for Conservation of Nature/Species Survival Commission [IUCN/SSC] Tortoise and Freshwater Turtle Specialist Group [TFTSG] and Asian Turtle Trade Working Group 2000), and one of the world's 25 most endangered turtles (Turtle Conservation Coalition 2018). The true locality of the species was later determined to be the Palawan island complex, to which it is endemic (Diesmos et al. 2004), and genetic analyses resulted in its re-assignment to the genus *Siebenrockiella* in 2005 (Diesmos et al. 2005). Large adult males reach a carapace length of 30 cm and weight of 3.5 kg, while adult females reach 21 cm and 1.4 kg (Diesmos et al. 2012). It is a semiaquatic, omnivorous, nocturnal species, residing in lowland forest streams (Diesmos et al. 2012). Females produce 1–2 eggs/clutch (Diesmos et al. 2012).

Upon its "rediscovery," despite protection under the Philippines Wildlife Resources Conservation and Protection Act of 2001 and Appendix II of the Convention on International Trade in Endangered Species (CITES), the species began to appear in the illegal wildlife trade, commanding high prices because of its rarity (Sy et al. 2020). Seizures of illegally collected specimens between 2005 and 2014 were sporadic, generally involving relatively small numbers of animals, but in 2015, 10 seizure events occurred, including one event involving 3831 individuals, many of which were in poor health (Devanadera et al. 2015; Schoppe et al. 2016; Sy et al. 2020). The general management and outcome of this confiscation have been previously described, including an 88% survival rate, with 3385 individuals repatriated to their natural range (Devanadera et al. 2015; Schoppe et al. 2016). Here we describe the veterinary management of these confiscated turtles, as well as subsequent use of biological specimens that were collected during the event.

METHODS

Details of veterinary observations and management of the confiscated turtles were retrospectively reviewed from several sources, including the personal journals and electronic communications of the authors, daily activity summaries and inventory lists created by rescue personnel during the course of the event, and reports that were later prepared for government authorities and funding agencies, grant proposals, and published summaries (Devanadera et al. 2015; Schoppe et al. 2016; Sy et al. 2020). Data and information were organized into categories of general management, physical examination and triage, morphometric data, hematologic specimens, parasitologic specimens, necropsy findings, therapeutic management, archived biological specimens, and capacity building. Details of the medical management of individual animals were not reviewed for purposes of this report; rather, we focus here on general methods that were applied to the majority of the animals. Biological specimens were exported from the Philippines to the United States for processing under CITES Permit No. 00001-A-2016 and PCSDS Wildlife Export Certificate No. 16-02.

Physical Examination and Triage. — Examinations were conducted by veterinarians with substantial experience in chelonian medicine. Examination included subjective assessment of weight, body condition, hydration, respiratory effort, and responsiveness, and examination of the oral cavity, nares, eyes, ears, skin, appendages, shell, and coelomic palpation. During initial evaluation, deceased turtles were removed, and live turtles were divided among 3 categories: grossly normal, mild to moderate illness, and severe illness. Each turtle was identified with a colored dot using nail polish on the carapace corresponding to their triage category. Turtles were reexamined approximately every 3 d and reassigned among categories based on serial examinations over time.

Morphometric Measurements. — Nine days after arrival at the rehabilitation center, morphometric measurements were acquired for 100 individuals that were selected to represent the breadth of body sizes among the group, but without consideration for selecting individuals from any specific triage category. Measurements were acquired with a scale, calipers, and soft measuring tape, including weight, straight and curved carapace length (SCL, CCL), straight and curved carapace width (SCW, CCW), straight plastron length (SPL), straight plastron width (SPW), and height. Sex was assigned using external sexually dimorphic characteristics, including greater tail length, more distal cloacal opening, and plastron concavity of adult males. Sex was not assigned to juvenile turtles that lacked clear dimorphism. Morphometric measurements, as above, were also collected postmortem from a subset of necropsy cases.

Hematology. — On the same day, blood was collected from 47 of the individual turtles that had been selected for morphometric measurements. Approximately 0.3 ml of

blood was collected from the external jugular vein using a 1-ml syringe and a 25-gauge needle. Immediately after collection, one drop of blood was used to prepare a blood film for future slide review, several drops of blood were transferred to FTA cards (Millipore Sigma, St. Louis, MO) for future genetics study, and remaining blood was used to fill capillary tubes for same-day determination of packed cell volume (PCV) and total protein (TP) using standard centrifugation and refractometry methods (StatSpin VT centrifuge, Beckman Coulter, Brea, CA), respectively. Blood film review was performed months later in the United States by a board-certified veterinary clinical pathologist (NIS) after slides were stained with Wright-Giemsa (Harleco®, EMD Millipore, Billerica, MA). Blood film evaluation included a white blood cell (WBC) estimate (Weiss 1984), a 200-WBC differential, blood cell morphological evaluation, hemoparasites (number of affected red blood cells [RBC] per 100 RBC), and subjective assessment of thrombocytes. Heterophil toxic change was evaluated on a scale of 1+ to 3+ (Stacy and Harr 2020).

Necropsy. — Gross necropsy was conducted in real-time for a limited number of deceased individuals that died over the course of the event in order to gain insight into pathologic processes that might inform the medical management of the remainder of the group, as well as to document gross anatomy and pathologic processes for this understudied species. Necropsies were conducted by a board certified veterinary pathologist (KC) and experienced veterinarians under field conditions using hand tools to remove the plastron (hammer, chisel, saw, hatchet), and standard dissection tools to evaluate the viscera. Later in the response, personnel who had been trained by the veterinarians also participated in necropsies (see "Capacity Building"). Aside from availability of personnel, and avoidance of turtles in poor postmortem condition, there was no specific method for choosing individual turtles for necropsy. Lesions were described and photographically documented, and selected tissues from each necropsy were preserved in 10% neutral buffered formalin (NBF) for later routine processing, embedding, and hematoxylin and eosin (HE) staining for later histopathologic analysis. A separate, more detailed report on these results is planned. During some necropsies, muscle tissue was preserved in ethanol for future genetic analyses. This species is poorly represented in scientific museum collections, so 4 deceased individuals were preserved intact as museum specimens by infusing the coelom with NBF, and then immersing the carcass in NBF. Additional samples collected at necropsy varied by case but included tissue imprints on Whatman FTA filter paper cards, external and internal parasites and feces (see below), and swabs or tissue in RNALater (Life Technologies, Grand Island, NY) for nucleic acid preservation. All samples were maintained at ambient temperature until export to the United States. Upon receipt in the United States, samples in RNALater and on filter papers were transferred to cold storage (−70°C to −80°C).

Parasitologic Specimens. — Grossly visible parasites that were detected during physical examinations and necropsies were preserved in 95% ethanol. Parasites were collected from 41 animals, most with mixed parasite species present from the colon, stomach, or jejunum. Fecal samples were collected from 24 necropsy specimens and stored in SAF fixative (Thermo Fischer Scientific, Waltham, MA). Parasitologic examination was conducted months later in the United States by a veterinary parasitologist (H.D.S.W.). Nematodes were transferred to 70% ethanol and cleared with lactophenol for morphological examination. Fecal samples were evaluated for the presence of parasites by sedimentation (Zajac and Conboy 2012). Ticks from 2 individuals were transferred to 70% ethanol and examined and identified.

RESULTS

General Management. — The Katala Foundation (KFI), Palawan, under the direction of one of the authors (S.S.), was contacted on 17 June 2015 (Day 1) by the Palawan Council for Sustainable Development Staff (PCSDS), when nearly 4000 live turtles were confiscated by PCSD law enforcement. KFI had been involved with in situ biological surveys and conservation of *S. leytensis* for several years prior to the confiscation. Arrangements were made to transfer the turtles by truck (Fig. 1) to the Palawan Wildlife Rescue and Conservation Center (PWRCC) for evaluation, at which they arrived the following day. Animals had been severely overcrowded, stacked many layers deep, and exposed to the sun. An initial census counted 3831 live and 60 dead *S. leytensis*, 168 live and 6 dead *Cyclemys dentata*, and 25 live *Cuora amboinensis*. Management of the latter two species was limited and will not be described further herein.

To initially accommodate the turtles, PCSDS acquired 4 truckloads of freshwater, with which 2 large concrete pools were filled with several centimeters of

Figure 1. Nearly 4000 Palawan forest turtles (*Siebenrockiella leytensis*) arrive for rehabilitation after confiscation from the illegal wildlife trade. Photo courtesy of S. Schoppe. (Color version available online.)
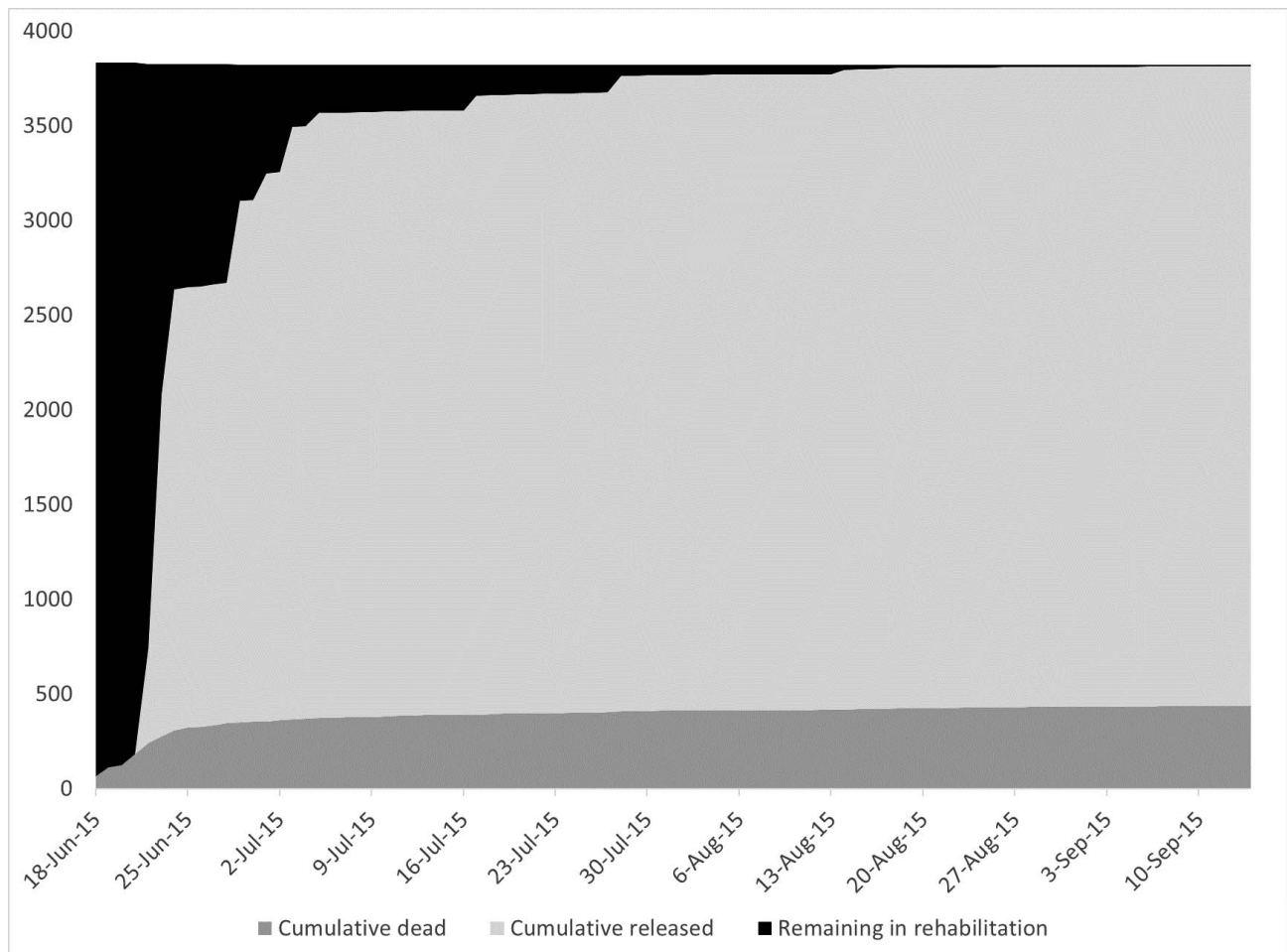
Figure 2. Graphical representation of the status of confiscated Palawan forest turtles (*Siebenrockiella leytensis*) over time. The majority of turtles were released (light gray) within several weeks as the number of turtles in rehabilitation (black) declined. The majority of deaths (dark gray) occurred within the first week.

water. Nonetheless, turtles remained very crowded. Acknowledging the magnitude of the situation and the poor health of many of the turtles, KFI put forth an international call for veterinary and husbandry assistance, supplies, and economic support. Many organizations and individuals pledged support (see "Acknowledgments"), and personnel were deployed to Palawan within days. Arrangements were made to relocate turtles on Days 3 and 4 within PWRCC to a series of shaded, vegetated enclosures that provided both terrestrial and aquatic habitat. Food was offered daily, including mainly water spinach (*Ipomoea aquatica*), which is eaten by the species in anthropogenically altered habitats such as rice fields and was available in large quantities. Over time additional food items included fruits, other vegetables, fish, liver, chicken, and mixtures of Oxbow Critical Care Diets (Carnivore and Herbivore; Oxbow Animal Health, Omaha, NE).

On Day 5, the IUCN/SSC TFTSG issued a recommendation that all turtles that did not clearly need extensive rehabilitative care should be released into the wild swiftly, ideally into areas that were previously inhabited by the species. This recommendation was based

on several factors, including the propensity of this species for intense intraspecific aggression, the fact that the turtles had not been exposed to nonnative species (thus reducing disease risk), and the fact that they were still on the same island from which they had been collected.

On Day 6, after the arrival of several veterinarians and establishment of a small outdoor veterinary clinic, veterinarians began to evaluate every turtle, separating them into 1 of the 3 triage groups. Approximately 2400 turtles appeared to be in good condition, were assigned to the grossly normal triage group, and were released by Day 8 with approval of PCSDS. Veterinary evaluation, treatment, and supportive husbandry continued over time, and turtles that were deemed to be in good health were released at sites selected by the KFI staff. International responders departed after 6 wks, and the care of 57 remaining turtles was continued by KFI personnel. Within 3 mo of the confiscation only 6 turtles remained under care, and after 8 mo the last 6 turtles were released. Overall, 440 turtles died during rehabilitation, and 6 escaped, with the majority of deaths occurring in the first 8 d after arrival at PWRCC (Fig. 2). In total 3385 individuals (88%) were repatriated to their natural range, as previously

Figure 3. Lesions seen during examination and treatment of confiscated Palawan forest turtles (*Siebenrockiella leytensis*). (A) Corneal edema of the right eye. (B) Corneal plaque on the right eye. (C) Corneal plaque sloughing after approximately 1 mo of treatment, revealing healthy cornea below. (D) Severe swelling of the left front foot associated with infection of the digits and loss of toenails. (E) Black discoloration and keratin flaking of the plastron belies underlying osteomyelitis. (F) Extensive necrosis of keratin and necrotizing osteomyelitis of plastron as revealed during debridement. Photos by P. Gibbons (A), C. J. Innis (B, D–F), and M. O'Connor (C). (Color version available online.)

described (Devanadera et al. 2015, Schoppe et al. 2016; Fig. 2). Postrelease monitoring of 3379 individuals over the subsequent 5 yrs recaptured 705 individuals (20.9%), of which 34.5% were captured repeatedly. For comparison, recapture rate of preexisting resident turtles during monitoring activities was 34.1% (S. Schoppe, unpubl. data, 2020).

Physical Examination and Triage. — Among ill turtles (mild to moderate illness and severe illness triage categories), ocular, skeletal, dermatologic, and behavioral

Table 1. Morphometric data for 100 confiscated Palawan forest turtles (*Siebenrockiella leytensis*) during their second week of rehabilitation. Data formatted as mean (median; range).

Variable[a]	Male (*n* = 29)	Female (*n* = 64)	Unknown sex juvenile (*n* = 7)
Weight (kg)	1.24 (1.1; 0.45–2.4)	0.93 (0.9; 0.47–1.7)	0.49 (0.45; 0.2–0.9)
SCL (mm)	214 (209; 150–287)	191 (190; 159–232)	155 (153; 119–193)
SCW (mm)	150 (150; 107–189)	134 (135; 113–155)	113 (113; 92–137)
CCL (mm)	222 (225; 115–297)	201 (199; 172–248)	163 (160; 125–202)
CCW (mm)	204 (205; 143–256)	187 (185; 154–288)	151 (151; 121–181)
SPL (mm)	186 (185; 79–247)	171 (172; 76–207)	155 (148; 110–220)
SPW (mm)	98 (89; 59–180)	81 (76; 66–180)	62 (64; 48–78)
Shell height (mm)	76 (75; 50–98)	73 (72; 63–94)	57 (58; 46–70)

[a] SCL and SCW = straight carapace length and width; CCL and CCW = curved carapace length and width; SPL and SPW = straight plastron length and width.

abnormalities were common (Fig. 3). Many turtles were subjectively underweight (i.e., the mass of the turtle felt subjectively low relative to its body size), and signs of sepsis were noted (e.g., plastron petechiae and ecchymoses). Ocular abnormalities included blepharoedema (swelling of the eyelids), conjunctivitis, keratitis (inflammation of the cornea), corneal ulcers, corneal edema, and corneal plaques (Fig. 3A–C). Cutaneous and skeletal abnormalities included ulcerative to necrotizing shell lesions, most commonly affecting the plastron (Fig. 3E–F), as well as swelling of the feet and digits, sometimes including partial or complete loss of individual digits (Fig. 3D). Other cutaneous lesions included ulcerative dermatitis, most commonly affecting the plantar and palmar surfaces of the feet, and multifocal erosion and loss of keratin of the shell, most commonly affecting the plastron. Shell lesions were often more extensive than they appeared externally, as described further herein under "Therapeutics" and "Necropsy". Behavioral abnormalities included generalized weakness (e.g., the head and limbs could be easily examined, the mouth could be easily opened), decreased responsiveness (e.g., decreased withdrawal into the shell, no attempt to bite the handler), and decreased mobility (e.g., turtles would not move to the water or shelter, but rather remain where they were placed). Abnormalities seen sporadically included pale mucous membranes, skin masses, and oral plaques, including lesions along the oral mucosa and tongue.

Veterinarians followed the progression of lesions over time, and the majority of lesions improved. Common signs of general improvement included subjectively increasing body mass, increased responsiveness (e.g., conspecific aggression, attempts to bite handlers), and return of normal behaviors (e.g., hiding, swimming, feeding). Swelling of the feet, digits, and eyelids resolved over time, and corneal, skin, and shell lesions healed. Signs of healing of the cornea and skin included re-epithelialization, fibrosis, eschar formation, and eschar sloughing (Fig. 3C).

Morphometrics. — Morphometric data collected on Day 11 for 100 individuals are summarized in Table 1.

Hematology. — Hematologic data collected on Day 11 for 47 individuals are summarized in Table 2. Leukocytes included heterophils, lymphocytes, monocytes, eosinophils, and basophils (Fig. 4). Leukocytosis

was common, with 45 of 47 individuals' WBC $\geq$ 30 K/μl, and was mainly characterized by heterophilia with left-shift, lymphocytosis (37 individuals $\geq$ 5.0 K/μl), monocytosis (37 individuals $\geq$ 3.0 K/μl), eosinophilia (10 individuals $\geq$ 3.0 K/μl), and basophilia (30 individuals $\geq$ 3.0 K/μl). Overall mild toxic changes of heterophils were noted for 6 turtles, including 1+ (*n* = 5) and 2+ toxic change (*n* = 1) in small numbers of cells. Toxic changes included mild degranulation, vacuolation, and/or slight cytoplasmic basophilia. Additional observations included the presence of plasma cells (*n* = 2), rare melanomacrophages (*n* = 2), erythrophagocytosis (*n* = 2), early erythrocyte precursor stages (*n* = 3), rouleaux formation (*n* = 3), mild anisocytosis (*n* = 20), mild polychromasia (*n* = 17), and variable numbers of hemogregarines (*n* = 40). Moderate anemia was seen in 17 individuals having PCV < 20%, moderate hypoproteinemia was seen in 9 individuals with total protein < 2.5 g/dl. Thrombocyte estimates were considered adequate for all turtles (i.e., presence of several thrombocytes per each high-power field in addition to small aggregates). Five samples had yellow plasma, and no samples showed evidence of hemolysis. Unfortunately, morphometric data could not confidently be paired with hematologic data because of several discrepancies in identification numbers; thus, correlations of hematologic data with morphometric data and sex could not be conducted.

Table 2. Hematologic data for 47 confiscated Palawan forest turtles (*Siebenrockiella leytensis*) during their second week of rehabilitation. RBC = red blood cells.

| Variable	Mean (median; minimum–maximum)
Packed cell volume (%)	22 (21; 15–37)
Immature RBC (no./100 mature RBC)	2.4 (0; 0–22)
White blood cell estimate (K/μl)	50.0 (40.4; 18.5–112.0)
Heterophils (K/μl)	24.9 (22; 6.1–63.0)
Immature heterophils (K/μl)	6.3 (3.9; 0.3–39.0)
Lymphocytes (K/μl)	7.3 (6.8; 2.8–16.0)
Monocytes (K/μl)	5.7 (4.4; 0.7–20.2)
Eosinophils (K/μl)	1.8 (1.2; 0.2–10.0)
Basophils (K/μl)	4.0 (3.4; 0.3–11.0)
Hemogregarines (no./100 RBC)	3.6 (3.0; 0–16.0)
Total protein by refractometer (g/dl)	3.4 (3.4; 1.0–5.2)
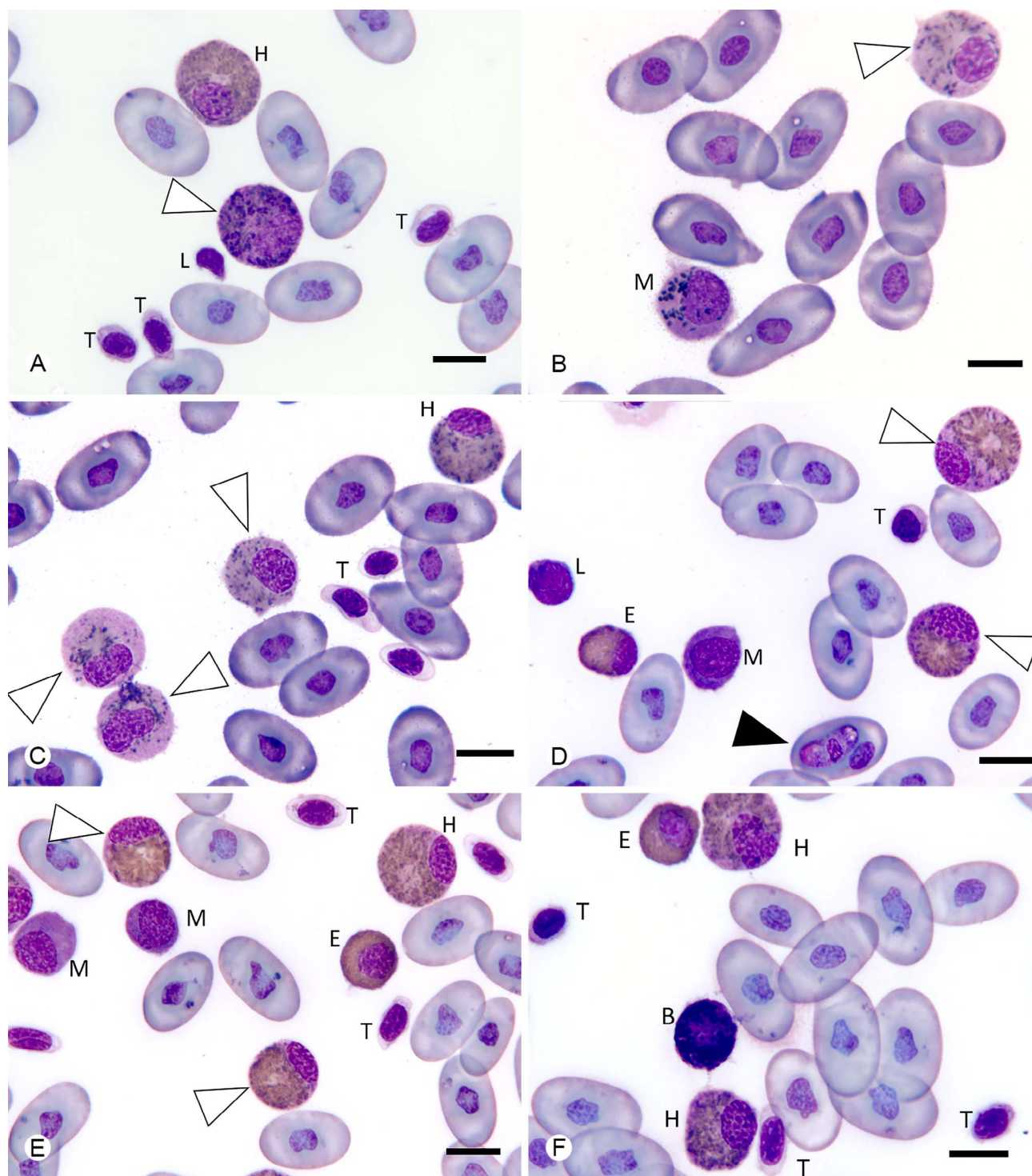
Figure 4. Photomicrographs of blood films of Palawan forest turtles (*Siebenrockiella leytensis*) showing leukocytes and thrombocytes, admixed with erythrocytes, some of which show small focal basophilic inclusions or small clear vacuoles, consistent with remnants of degenerate organelles. ×100 objective, scale bar = 10 μm, Wright-Giemsa stain. (A) Mature heterophil (H), immature heterophil (white arrowhead), small lymphocyte (L), thrombocytes (T). (B) Mildly toxic (1+), mature heterophil (white arrowhead), melanomacrophage (M). (C) Mildly toxic (1+) to moderately toxic (2+), immature heterophils (white arrowheads), mature heterophil, thrombocytes (T). (D) Mildly toxic heterophils (1+, white arrowheads), eosinophil (E), lymphocyte (L), monocyte (M), thrombocyte (T), erythrocyte with hemogregarine gametocyte (black arrowhead). (E) Mature heterophil (H), immature heterophils (white arrowheads), eosinophil (E), monocytes (M), thrombocytes (T). (F) Mature heterophils (H), eosinophil (E), basophil (B), thrombocytes (T). Photos by N. I. Stacy. (Color version available online.)

Figure 5. General anatomy of a female Palawan forest turtle (*Siebenrockiella leytensis*) at necropsy. The animal is in dorsal recumbency, head toward the top of the image, plastron removed. This individual measured 165 mm straight carapace length (SCL). No. 15 scalpel blade is 37 mm length. O = right and left ovaries; Li = liver; Lu = caudal extent of the right lung; S = greater curvature of the stomach. Note ulcerative pododermatitis of forelimb digits (arrows). Photo by C. J. Innis. (Color version available online.)

Parasitology. — Helminths recovered from 41 animals were most commonly a mixture of species including larval ascarids, *Falcaustra* spp. (commonly *F. annandalei, F. japonensis,* and *F. duyagi*), *Tonaudia* spp., and *Monohysterides testudinicola*. Eggs of *Falcaustra, Tonaudia,* or *Monohysterides* were recovered from 10 samples by fecal sedimentation. Ticks from 2 animals were identified as *Amblyomma geoemydae*.

Necropsy. — Available records indicated that ≥ 123 necropsies were performed throughout the event, with 45 necropsies taking place within the first 3 wks. Tissues were preserved in formalin for 115 necropsy cases, 141 samples from 91 turtles were collected in RNA Later, and 71 tissue imprints were collected on filter paper from 37 turtles. For this report, detailed necropsy reports including histopathologic findings were reviewed for 37 cases, all of which died within the first 2 wks (range = Day 7–14, median = Day 9, mean = Day 10), and for which necropsy was conducted within 24 hrs (*n* = 33) to 48 hrs (*n* = 4) of death. Thirty-two of the 37 cases originated from the severely ill triage group, while 5 originated from the mild to moderate illness triage group. Findings from additional cases will be reported elsewhere.

Anatomic features and viscera were typical of other chelonian species, in general (Figs. 5 and 6). Ovaries

Figure 6. Gonads of Palawan forest turtles (*Siebenrockiella leytensis*). (A) Postmortem coelioscopic view of immature ovary acquired at the Museum of Comparative Zoology, Harvard University. This individual was 147 mm straight carapace length (SCL). (B) Testes from two subadult individuals of similar body size at necropsy. The yellow testes are from an individual of 157 mm SCL, while the larger brown testes with developed epididymis are from an individual of 153 mm SCL. Scale bar in B shows 1-mm increments. Photos by C. J. Innis. (Color version available online.)

showed various states of follicular development. Mature females generally had one follicle that was larger than the remainder, consistent with the species' typical production of a single egg per clutch (Fig. 5), while immature ovaries showed typical follicular structure with no enlarged follicles (Fig. 6A). Testes varied from uniform yellow to striated brown–tan, ranging from several millimeters to 1 cm in diameter, and the epididymis was variably developed even among individuals of similar carapace length (Fig. 6B).

A slight skin depression, as observed in the past by KFI researchers (S. Schoppe, *pers. obs.*), was noted in all specimens on the ventral midline aspect of the tail, cranial to the vent, directly adjacent to the plastron margin. Grossly, this structure, referred to here as the precloacal fossa, was a discrete, ovoid, up to 5-mm-wide and 3-mm-long, slight indentation (Fig. 7). On cut section, there was mild thickening of the epidermis, and the interior rarely contained a waxy substance. Histologically, this structure contained a narrow invagination of the epidermis into the dermis, which was demarcated from the surrounding tissue
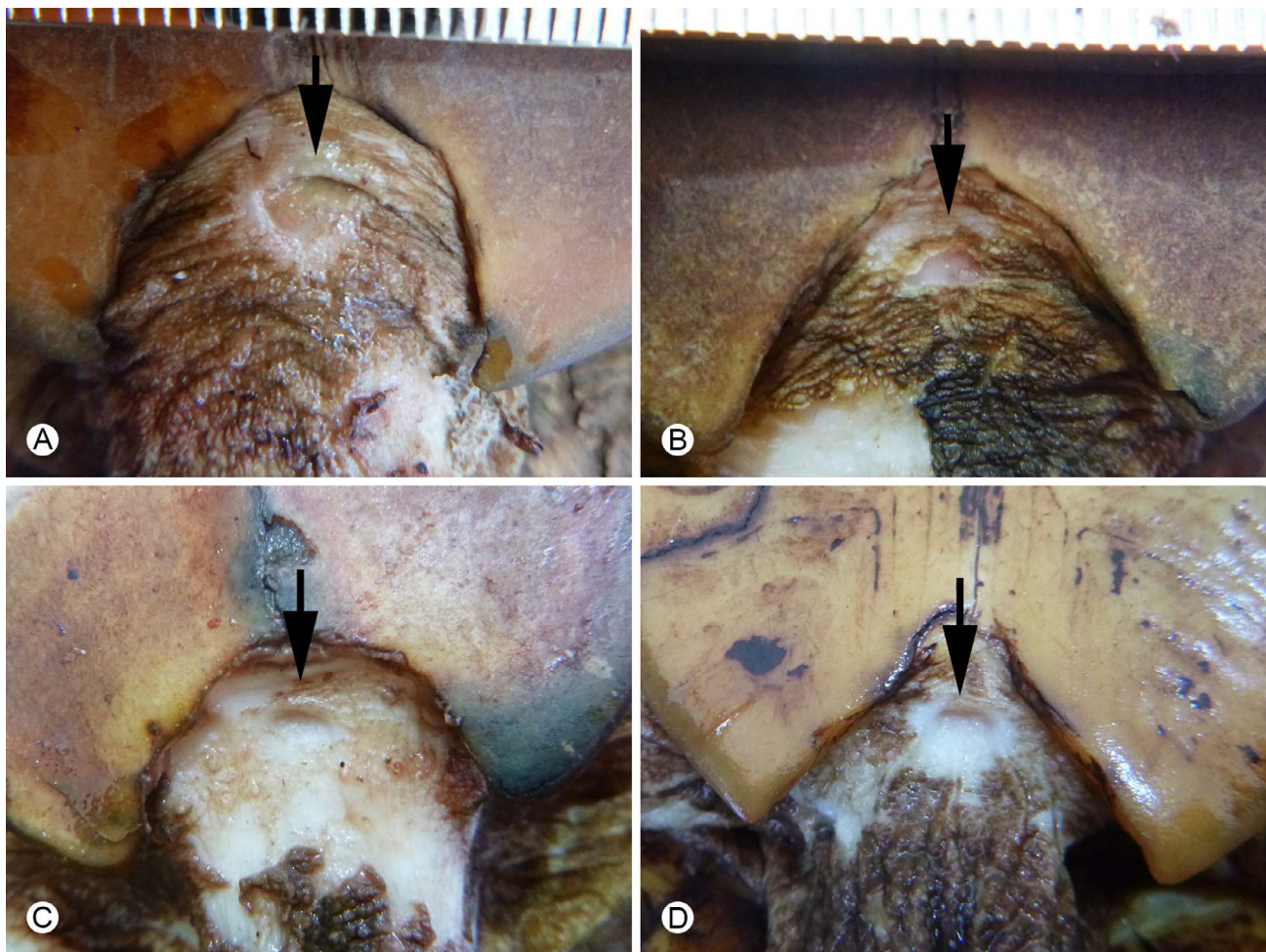
Figure 7. Ventral proximal tail of 4 Palawan forest turtles (*Siebenrockiella leytensis*), showing the precloacal fossa, a slight indentation directly caudal to the caudal margin of the plastron. (A) Male, 231 mm straight carapace length (SCL). (B) Female, 183 mm SCL. (C) Female, 165 mm SCL. (D) Male, 178 mm SCL. Photos by K. Conley. (Color version available online.)

by its finer collagen fibers, increased accumulation of lymphocytes in low to moderately dense aggregates, and increased vascularity with numerous capillaries and arterioles, the latter exhibiting an amphophilic, hyaline-like change (Fig. 8). Neither secretory material nor discrete glandular tissue were present.

For the 37 animals with detailed gross necropsy reports, the most commonly identified lesions included dermatitis and osteomyelitis of the shell (carapace, plastron, and/or bridge, Fig. 3), pododermatitis (Fig. 3), verminous colitis due to nematodiasis (Figs. 9 and 10), and soft tissue edema. Fat stores were reduced in 32 of these turtles, with no adipose stores identified in 15 of them.

Shell lesions were present in 36 of 37 cases, with gross lesions varying from areas of scute loss to full-thickness bone defects (Fig. 3). Histology of bony lesions was limited to 7 of the more severe cases, all of which revealed bony necrosis accompanied by granulocytic to granulomatous inflammation, variable bacterial presence, and occasional necrotic bony sequestra. Plastron lesions were often more severe than those on the carapace,

although carapacial disease occasionally predominated. Frequently affected sites on the carapace included the dorsal midline, the paravertebral areas (costal scutes), and the margins of the shell. On the plastron, external lesions often emanated from seams between scutes. When lesions were located in the center of scutes, they were often overlying the pectoral or pelvic girdle, and in multiple cases the inflammation of the shell and accompanying infection extended into the pelvic musculature, as identified grossly and confirmed histologically. In some animals, what appeared to be a minor superficial lesion revealed marked underlying osteomyelitis and bone necrosis, characterized by complete absence of bone, or extreme softening of bone. Some turtles exhibited osteomyelitis of the shell that ruptured or expanded internally toward the coelom with no external evidence of disease.

Pododermatitis was present in 36 of 37 cases (Fig. 3). Pododermatitis lesions consisted of sloughing of the superficial aspects of the skin with varying degrees of claw loss and less frequently digit loss. Necroulcerative dermatitis and bacterial infection were confirmed in the 6
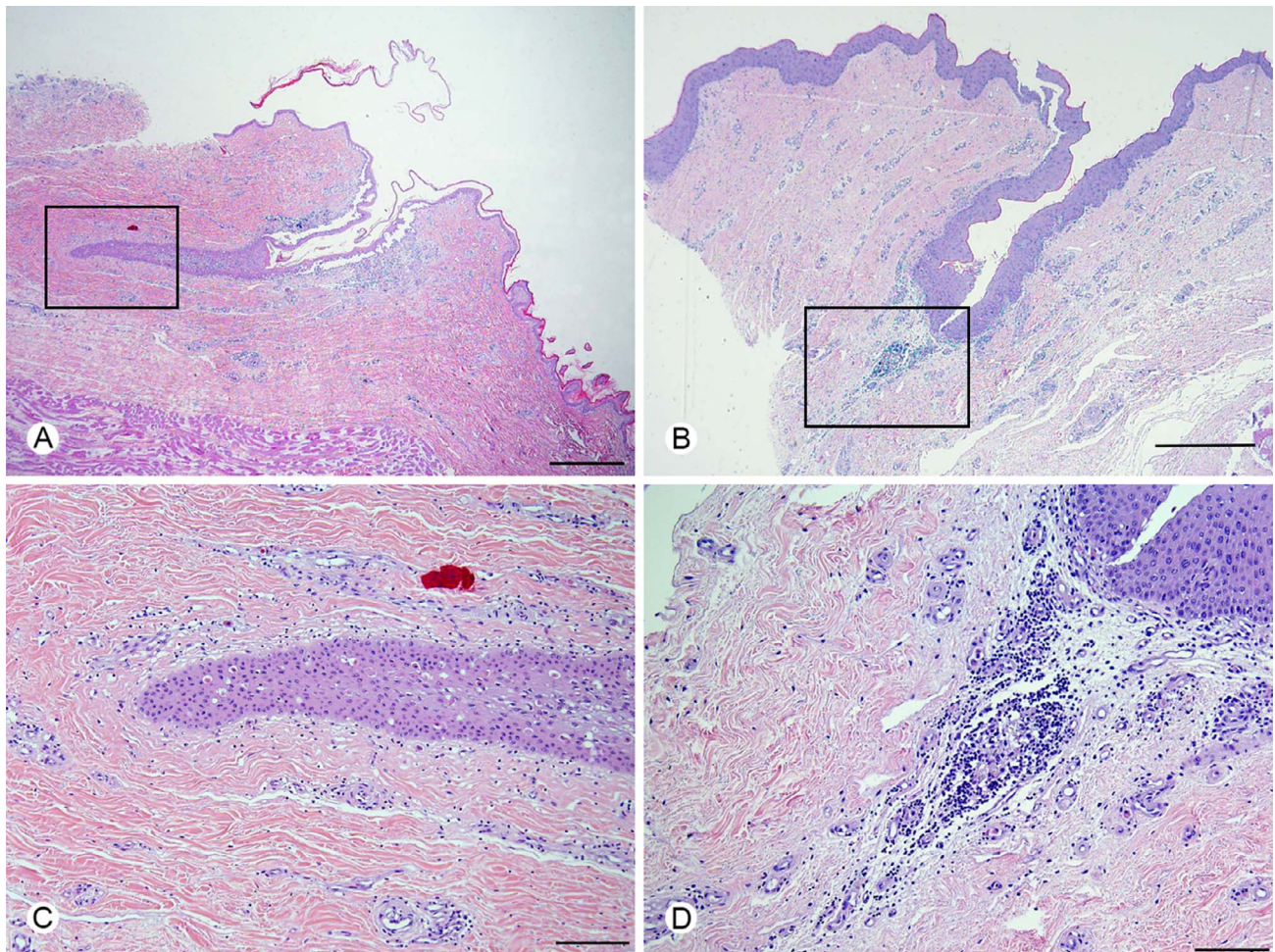
Figure 8. Histologic images of the ventral proximal tail from 2 Palawan forest turtles (*Siebenrockiella leytensis*), showing an invagination of epidermis in the area of the precloacal fossa. (A, C) Male, 178 mm straight carapace length (SCL). (B, D) Female, 165 mm SCL. (C, D) Higher magnification images of the insets in A and B. Note the pale staining of the dermis surrounding the invagination, the result of finer collagen fibers, as well as an increased population of lymphocytes, and vascularization. Hematoxylin and eosin stain. (A, B) 40× magnification, scale bar = 500 μm. (C, D) 200× magnification, scale bar = 100 μm. Photos by K. Conley. (Color version available online.)

cases examined histologically, some with associated osteomyelitis and confirmed phalanx loss. Lesions were more severe on the forelimbs than the hind.

Nematodes were identified in the large intestine of 36 of these cases, 35 of which also had grossly apparent inflammation of the large intestine. Inflammatory lesions were restricted to the proximal portion of the large intestine in all cases. They ranged from pinpoint intramural granulomas to marked thickening with innumerable large intramural granulomas (Fig. 9). The histologic presentation paralleled the gross findings, exhibiting variation in both severity and chronicity (Fig. 10). Predominant lesions included necrosis, heterophilic granulomas, fibrosis, ulceration, edema, and the presence of few to large numbers of viable and degenerating nematodes. The vast majority of nematodes identified histologically in colonic lesions were of the same morphology and were interpreted as likely *Falcaustra* species based on comparison with the more thoroughly examined whole specimens recovered at necropsy. A second morphologic type was found almost exclusively in

tissues other than the colon, most often in the gastric wall; these were not definitively identified.

Soft tissue edema, characterized histologically by increased clear space and dilated lymphatic vessels, was identified in 34 cases, all via histologic examination.

Trematodiasis consistent with schistosomiasis was present in 23 cases and was considered to be an incidental finding. It was characterized by trematode eggs or egg fragments embedded in tissue, with or without an associated granulomatous response; intravascular trematodes were rarely identified. Soft tissue mineralization was present in 11 cases, most often affecting the gastrointestinal tract (esophagus through cloaca) and the cardiovascular system. When severe, it was associated with variable inflammation, ulceration, edema, and in the gastrointestinal tract, secondary bacterial infection. Mineralization was only identified histologically; gastrointestinal tissues with confirmed mineralization were interpreted as pure inflammatory lesions grossly (thickened walls with roughened to corrugated and brown discolored mucosa). Five turtles had significant hemorrhage between the shell and coelomic
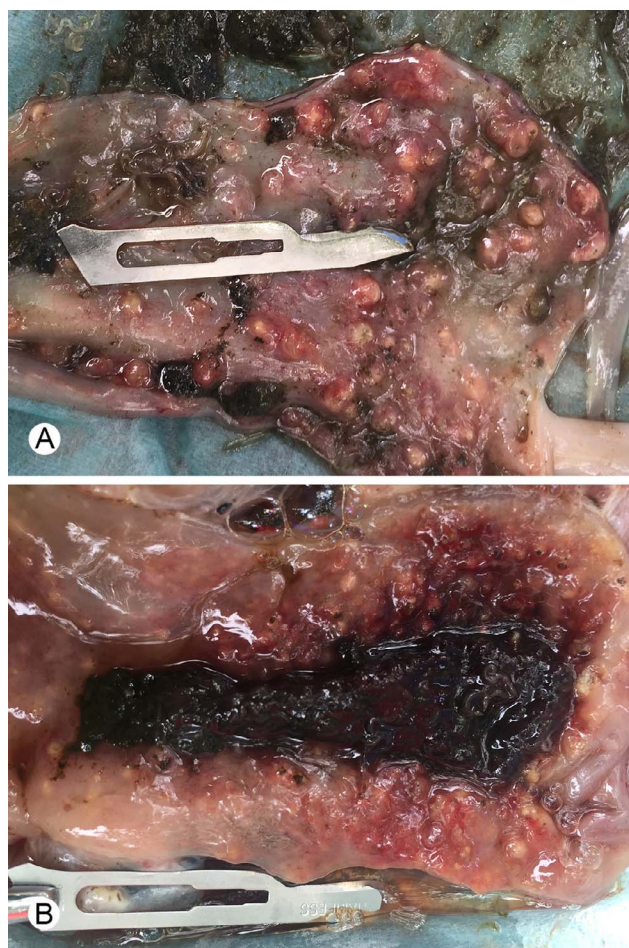
Figure 9. Gross postmortem view of the proximal colon mucosal surfaces from 2 Palawan forest turtles (*Siebenrockiella leytensis*). (A) Severe multifocal granulomas (yellow to pink nodules). (B) Severe multifocal granulomas and extensive hemorrhage. No. 15 scalpel blade is 37 mm in length. Photos by C. J. Innis. (Color version available online.)

membrane, 4 of which also had full-thickness or internal osteomyelitis lesions in close proximity to areas of hemorrhage; no such lesions were present in the fifth case. Hemorrhage was not further evaluated histologically.

There was no histologic indication of viral disease in any of the animals examined. Infection by fungi or fungal-like organisms was identified in 3 of the 37 cases: 1 with fungal pneumonia, 1 with fungal pleuritis, and 1 case with hyphal-like structures within ulcerative stomatitis lesions (possible oomycete or water mold). Protozoa were rare, with only 1 of the 37 animals with detailed necropsy reports containing amoeba. In this case, protozoa were present within an area of colitis and may have represented a secondary infection rather than a primary cause for inflammation, which was otherwise attributed to nematodiasis.

Therapeutics. — Medical supplies were donated to the event from numerous international sources (see "Acknowledgments") and were delivered to Palawan by arriving personnel or by international shipment. On Day 6, all turtles that were not in the "grossly normal" triage group were divided into 2 groups of roughly equal size, and each group was examined and medically managed once every 3 d. That is, one group of approximately 500–600 turtles was treated each day, while the third day was used for ongoing evaluations and other activities. Based on physical examination findings, prior experiences with confiscated turtles, and acknowledging the large number of turtles that required care, veterinarians established a practical and efficient treatment plan to provide nutritional support, rehydration, and treatment for bacterial and parasitic infections. Nutrition support and hydration were provided by tube feeding at approximately 20 ml/kg every 3 d. A variety of products were used based on availability, including electrolyte and glucose solutions (e.g., Re-Sorb, Zoetis, Parsippany, NJ), and enteral feeding formulas (e.g., Critical Care Herbivore, Critical Care Carnivore, Oxbow Animal Health, Omaha, NE; Emeraid, LaFeber Vet, Cornell, IL). Antibiotic therapy was provided using ceftazidime at 20 mg/kg intramuscularly every 3 d (a widely used dose and frequency for chelonians, e.g., Stamper et al. 1999). Turtles that received antibiotics were treated ≥ 3 times to provide a reasonable minimum duration of treatment (approximately 10 d), and treatment was extended as needed based on serial examinations.

Antiparasitic therapy was administered on the second and third treatment cycle (Days 9 and 10, Days 12 and 13) based on previous experience with other confiscated turtles and supported by necropsies that showed colonic lesions associated with nematodes, as well as concern for amoebiasis as a common cause of chelonian colitis (Innis et al. 2006; Rivera et al. 2009). Metronidazole (crushed tablets) and fenbendazole (solution) were added to the oral electrolyte solution to achieve approximate doses of 25 mg/kg for metronidazole and 50 mg/kg for fenbendazole. These doses were selected based on the combined clinical experience of the involved veterinarians, professional judgment, and limited pharmacokinetic data for other chelonian species (e.g., Innis et al. 2007). Turtles treated on Day 13 did not receive metronidazole as the supply had been exhausted.

During each treatment cycle, eye lesions were treated with a topical ophthalmic antibiotic ointment or solution (available options included commonly used ophthalmic products such as neomycin–polymxin–gramicidin, ofloxacin, tobramycin, oxytetracycline, and gentamicin). Shell lesions were treated by debridement, cleaning, and topical therapy. Debridement was generally achieved using a conventional dental scaler (i.e., not an ultrasonic scaler) to gently remove loose keratin, caseous debris, and necrotic bone, taking care to avoid aggressive debridement in the absence of general anesthesia. Affected sites were then cleaned with a topical disinfectant (e.g., povidone iodine or chlorhexidine), and coated with a topical antibacterial product (e.g., silver sulfadiazine cream, neomycin/polymyxin/bacitracin ointment).

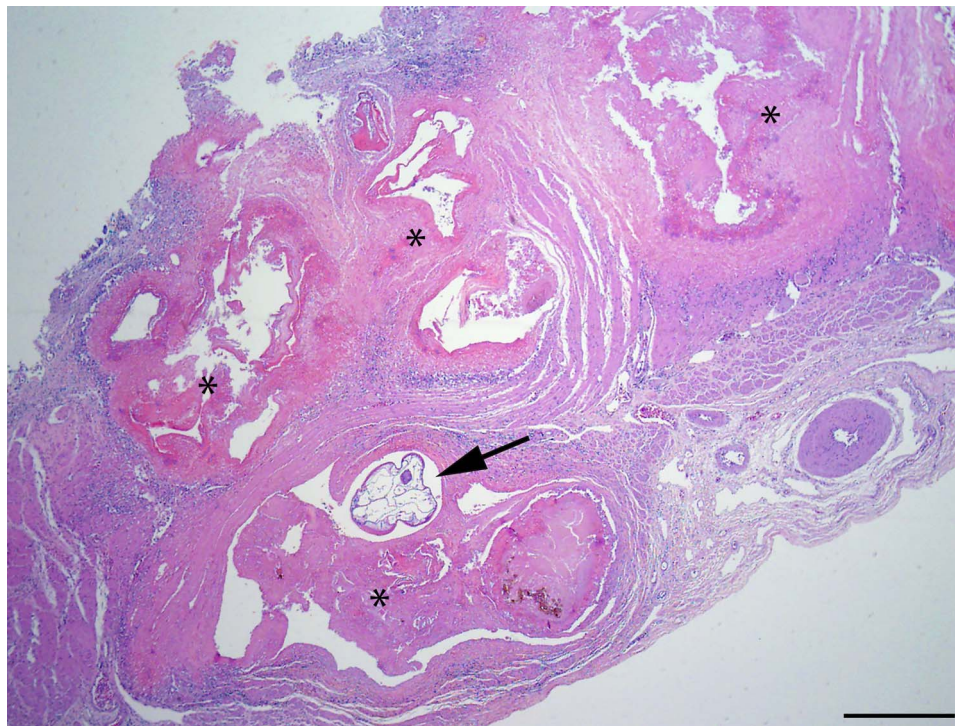After Day 13, therapy was withheld if deemed unnecessary, while treatment continued for turtles that

Figure 10. Histologic images of the colon of Palawan forest turtles (*Siebenrockiella leytensis*) affected by verminous colitis. Bright pink areas (asterisks), effacing much of the normal colonic wall, are areas of necrosis and inflammation. A nematode is present in the center of one such lesion (arrow). The mucosal surface is at the top right of the image and the serosa at the bottom left. Hematoxylin and eosin stain, 40× magnification, scale bar = 500 μm. Photo by K. Conley. (Color version available online.)

remained ill. Within 3 wks, approximately 300 turtles continued treatment, mainly because of ongoing shell and eye lesions. The majority of shell debridement was discontinued by Day 19 (5 July) because most lesions showed evidence of healing.

Archived Biological Specimens. — A variety of biological samples were exported for further evaluation and specimen archives. Exported blood smears and parasitological samples were evaluated as described in this article. Formalin-fixed tissue suites that were collected at necropsy were transferred to the veterinary pathology laboratory of the Wildlife Conservation Society, Bronx, New York, for histopathologic evaluation. Muscle samples ($n = 34$) and blood samples ($n = 125$) were archived at the Shaffer Laboratory, Department of Ecology and Evolutionary Biology, University of California, Los Angeles, for future genetic studies. Four complete carcasses were exported; 2 were deposited in the collection of the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, Massachusetts (MCZ R-194534, R194535) and 2 in the collection of the American Museum of Natural History, New York, New York (R178790, R178791). MCZ specimens were later evaluated by laparoscopy for examination of the gonad to identify sex, which was not externally evident; both specimens were female (Fig. 6A).

Capacity Building. — International veterinary and husbandry staff trained many Philippine students, KFI personnel, and several Philippine veterinarians throughout the event, with hope of increasing the capacity for local intervention in future confiscations in the absence of international personnel. In addition, international staff provided consultation to KFI regarding enclosure design, water quality, and general husbandry. KFI trained numerous local students in postrelease monitoring, 5 of which used the research for their respective undergraduate thesis.

DISCUSSION

Repatriation of confiscated wildlife is a complicated process, carrying substantial risk (IUCN 2019). Repatriated animals may fail to survive, they may introduce disease, or they may introduce deleterious genetic material (IUCN 2019). Guidelines and decision-tree analyses exist for the management of confiscated live organisms, in general, including limited specific recommendations for chelonians (Jacobson et al. 1999; IUCN 2019; Raphael 2019). In the case of the 2015 Palawan forest turtle confiscation, the IUCN Tortoise and Freshwater Turtle Specialist Group and PCSDS made a rapid decision to repatriate as many animals as possible as quickly as possible, with consideration of the medical status of each individual. This decision was made with concern for preventing extinction of the species resulting from the removal of nearly 4000 individuals from the population. Aspects that favored repatriation included the animals being confiscated on the same island from which they were collected; they were not mixed with nonnative species;

there was an established in situ research, conservation, and monitoring program for the species; adequate habitat existed; and severe intraspecific aggression would preclude long-term holding of large numbers of individuals. Although additional long-term observations are required to monitor for deleterious effects, the first 5 yrs of postrepatriation monitoring indicate that a substantial number of individuals survived.

Veterinarians play a key role in the management of confiscated turtles, providing medical evaluation and medical care, conducting disease surveillance, participating in risk analyses, and documenting their findings and outcomes (Innis et al. 2002; Rivera et al. 2009; Raphael 2019). Veterinary investigations of confiscated animals have elucidated novel disease processes, novel parasite species, and helped to establish a baseline of biomedical information for many chelonian species (Innis et al. 2002, 2006; McCallum et al. 2011; Ward et al. 2012; Raphael 2019). Yet, few reports have documented veterinary observations that occurred during large confiscation events, and none of the available reports have provided comprehensive veterinary observations as reported here. Establishment of biomedical data for healthy turtles in wild populations can provide a baseline to distinguish between recently collected, relatively healthy wild turtles that are ready for release and unhealthy turtles that will not be releasable or need additional rehabilitation before release (McCallum et al. 2011; Henen et al. 2013). The present study is an excellent example of the breadth of biomedical information and biological specimens that can be acquired, documented, and archived during confiscation events. These data can inform future veterinary management of this species, and also prove illustrative for other large-scale confiscations. Given the number of turtles and limited personnel, we were unable to create individual medical records for each turtle, and thus the prevalence of specific disease conditions cannot be determined.

Although generally successful, this event required a large amount of resources, including substantial donation of personnel, travel and lodging costs, and supplies. This model of acute, international resource deployment may not be practical or sustainable in the future. There is ongoing need for training of range-country personnel in the medical evaluation, treatment, and general management of confiscated chelonians. During the Palawan event, many local personnel, including students, were trained in examination, treatment, and necropsy techniques, such that they are well poised to manage future confiscations with lesser international involvement.

Performing necropsies concurrently with veterinary triage and husbandry support was an important component of this event. Real-time postmortem examination provided information to the clinical and husbandry teams regarding case management and predominant disease processes. For example, the internal severity of many of the shell lesions was first appreciated at necropsy, leading to more aggressive management of those lesions in the remaining live animals. Similarly, the extent and severity of colonic nematodiasis that was first diagnosed during necropsies led to specific anthelminthic therapy for the remaining live animals. The presence of a veterinary pathologist whose primary responsibility was to perform postmortem examinations allowed the clinical and husbandry teams to focus on treatment and management of surviving animals and provided an opportunity for in-country capacity building. In addition to describing pathologic processes, necropsy and histologic evaluation provided insight into the basic anatomy of this species, including what appears to be a unique anatomic feature, the so-called precloacal fossa. While the function of this structure remains unclear, we are hopeful that investigation of additional tissues collected from this event will yield more information.

Disease conditions seen during this event were typical of those seen in turtles in the wildlife trade, including dehydration, various types of infections, and poor body condition. The most frequent and severe disease processes in the confiscated turtles included inflammation affecting the shell and feet, large intestinal nematodiasis, and loss of body condition.

Shell diseases are commonly encountered in wild and captive chelonians and can be the result of multiple etiologies. Infection (primarily bacterial or fungal), trauma, and metabolic bone diseases (primarily nutritional secondary hyperparathyroidism) are most commonly identified (Meyer and Selleri 2019). In nontraumatic cases, common concurrent factors in chelonians with shell disease include poor water quality, abrasive substrates, improper temperature ranges, poor nutrition, overcrowding, and insufficient basking and/or ultraviolet light exposure (Barten 2006). In the confiscated forest turtles, the location of shell lesions may suggest causality. Those in areas of ground contact—the pelvic and pectoral plastron lesions—could have been the result of excessive downward pressure from stacking turtles on top of each other, or extensive time spent out of water on unnatural substrates. Lesions emanating from seams between scutes suggest impaired physical barriers, potentially resulting from poor environmental quality (e.g., excessive fecal and urine exposure). Finally, there were some intraosseous and coelomic membrane inflammatory lesions, which were not associated with any discernible defects in the overlying keratin. A cause for lesions presenting without evidence of external trauma was not specifically identified. Potential causes include, but are not limited to, systemic bacterial showering of the bone from distant sites of infection (e.g., colon or feet), local infarction related or unrelated to schistosomiasis, and secondary to penetrating or external trauma that had since healed. Increased pressure in specific sites due to stacking of animals and associated tissue trauma or hypoxia may have primed such locations for infection during periods of bacteremia, as seen in several pelvic bone lesions that lacked associated plastron keratin defects. Marked hemorrhage between the shell and underlying coelomic membrane, interpreted to be a

significant contributor to mortality in these cases, was thought to be secondary to severe osteomyelitis in 4 of the 5 cases in which it was identified. Trauma, in addition to infection or as the sole insult in the fifth case, was another possible explanation, although histology was not performed on hemorrhagic regions and a specific cause was not elucidated. Similarly, pododermatitis was presumably related to housing conditions prior to confiscation. Inappropriate and abrasive substrate, excessive humidity, and secondary infections have been reported to cause foot lesions in chelonians (Scheelings and Hellebuyck 2019).

Ophthalmic disorders, including corneal lesions, are commonly diagnosed in chelonian patients. Common causes include vitamin A deficiency, infectious agents (viral, bacterial, fungal), trauma, exposure to extreme temperatures, and exposure to excessive ultraviolet light (Lawton 2019). Corneal lesions in the confiscated turtles were often severe, resulting in nearly complete corneal opacity. The pathogenesis of these lesions was likely multifactorial, possibly involving poor environmental conditions, dehydration, exposure to urine and feces while densely packed, exposure to atypical amounts of direct sunlight while removed from their habitat, and secondary infection due to reduced immune response. No specific bacterial, fungal, or viral agents were identified in these cases, but such diagnoses typically require microbiological and molecular diagnostic methods that were not available during this field response. It is possible that insight may be gained from future analyses of archived specimens. Despite the severity, the majority of corneal lesions resolved over time by eschar formation, healing of the deeper corneal epithelium, and eventual eschar sloughing (Fig. 3). It is likely that improved environmental conditions, decreased crowding, and supportive therapy promoted healing.

Colonic nematodiasis produced some of the more striking gross and histologic lesions among these cases and was likely a primary driver of morbidity and mortality. Colitis due to nematodes has been documented as a cause of illness and death in several chelonian species (Rideout et al. 1987; Innis et al. 2006). Some turtles had only few granulomas, but many had extensive chronic and ongoing inflammatory lesions that extended deep into the wall of the colon, and secondary infections were common. The majority of nematodes collected at necropsy were identified as *Falcaustra* spp. Morphologic similarities between the intact specimens collected at necropsy and those seen in colonic lesions histologically suggested that the latter were also of the *Falcaustra* genus. Disease and lesions associated with *Falcaustra* spp. (or the Kathlaniidae more broadly) in reptiles are not well-described, despite numerous herpetofaunal host reports (e.g., Bursey and Rivera 2009). Indeed, *Falcaustra* spp., along with the other species identified, represent common nematodes from turtles of this geographic area (Purwaningsih and Mumpuni 2015). It is possible that these nematodes are truly primary pathogens for *S. leytensis*. However, given the poor environmental conditions in which these turtles were held prior to confiscation and the presumed long duration of time required to harvest such a large number of individuals, it is likely that environmental factors, prolonged deprivation of food and water, and chronic stress likely played a role in disease severity. Parasitologic investigation into the wild population of *S. leytensis* may provide more information regarding the pathogenicity of these nematodes.

Metastatic soft tissue mineralization in reptiles results from derangements of calcium and phosphorus metabolism, which occur in a variety of situations including renal disease and secondary hyperparathyroidism, hypervitaminosis D, and other dietary imbalances and osteolytic bone disease (Graham et al. 2020). Most of the affected turtles did not have overt renal disease, so other conditions were considered more likely, including "paradoxical metastatic mineralization" occurring in reptiles with normal or low calcium and/or vitamin D levels. Soft tissue edema can similarly be related to suboptimal care with starvation or protein deficiency resulting in hypoproteinemia followed by edema. The severity of colonic disease could have also contributed to edema development in many cases, with loss of protein through the severely damaged colon.

Schistosomiasis is a common finding in aquatic turtles (Johnson et al. 1998). Mild infections are often considered an incidental finding, but severe infections can cause morbidity and mortality. These organisms colonize the cardiovascular system, releasing eggs into the vasculature, which eventually become embedded in capillaries and small vessels and incite an inflammatory response. If this occurs in large vessels or areas with little collateral circulation, tissue infarction can occur. In the confiscated *S. leytensis,* schistosomiasis was minimal or mild.

The tick, *A. geoemydae,* is commonly found on other turtle species in southeast Asia (Simmons and Burridge 2000), but to our knowledge their presence on *S. leytensis* represents a new host record. Ticks have not commonly been noted on this species during field surveys (S. Schoppe, *pers. obs.*). The relevance of ticks as possible disease vectors for this species requires further study.

Blood data were consistent with clinical observations and necropsy findings, showing evidence of chronic-active inflammation, including extreme leukocytosis, heterophilia, left-shifted and/or toxic heterophils, and monocytosis. Although data for healthy individuals of this species are lacking, even the lowest WBC concentrations in the confiscated turtles were above the WBC ranges for healthy individuals of other species of the family Geoemydidae (Hidalgo-Vila et al. 2007; Chung et al. 2009; Chansue et al. 2011). Mean PCV and TP of confiscated turtles were similar to those described for related species, but some confiscated turtles showed evidence of anemia and hypoproteinemia, and others showed evidence of hemoconcentration (i.e., elevated PCV and TP). It is probable that hemoconcentration due to dehydration masked anemia and hypoproteinemia in some cases. Nonregenerative

anemia in some turtles was presumably associated with inflammatory disease. Further evaluation of the physiologic status of the confiscated turtles would have been informative and interesting but was beyond available analytical and financial abilities.

Although tragic, this confiscation provided an opportunity to learn more about the biology of this understudied, critically endangered species, and the investigators sought to collect and disseminate biological materials to appropriate locations. The physical examination, clinical pathologic, parasitologic, and histopathologic data described here indicate that confiscated turtles may be affected by a variety of disease conditions of varying severity, including inflammatory and necrotizing disease states, with evidence of bacterial and parasitic lesions in many individuals. These findings highlight the importance of veterinary involvement in such confiscations, providing therapeutic and husbandry management to restore hydration and nutritional status, reduce stress, treat infectious agents, and promote healing of damaged tissues. Assignment of ill turtles into triage groups was helpful, despite treating both illness groups similarly, because it allowed clinicians to recognize the history of each turtle when making decisions about their suitability for release. That is, turtles that were at any time assigned to the severe illness triage group were scrutinized a bit more during later evaluations. The groupings also allowed clinicians to make some assessment of progress or decline as turtles moved between triage groups over time.

In addition to the clinical, pathologic, and parasitologic data provided here, additional samples remain available for future molecular diagnostic and genetics studies, and intact specimens are now available for the first time in 2 major American museum collections. The authors encourage similar efforts during future postconfiscation efforts for endangered chelonians.

CONCLUSIONS

An international effort to triage, treat, and repatriate nearly 4000 confiscated, endangered Palawan forest turtles was largely successful, and substantial novel biomedical data were documented. Future efforts during similar chelonian confiscation events should seek to document clinical findings and thoroughly use available biological materials. Long-term monitoring of repatriated specimens is encouraged to inform and refine our understanding of the impact and outcome of such events.

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