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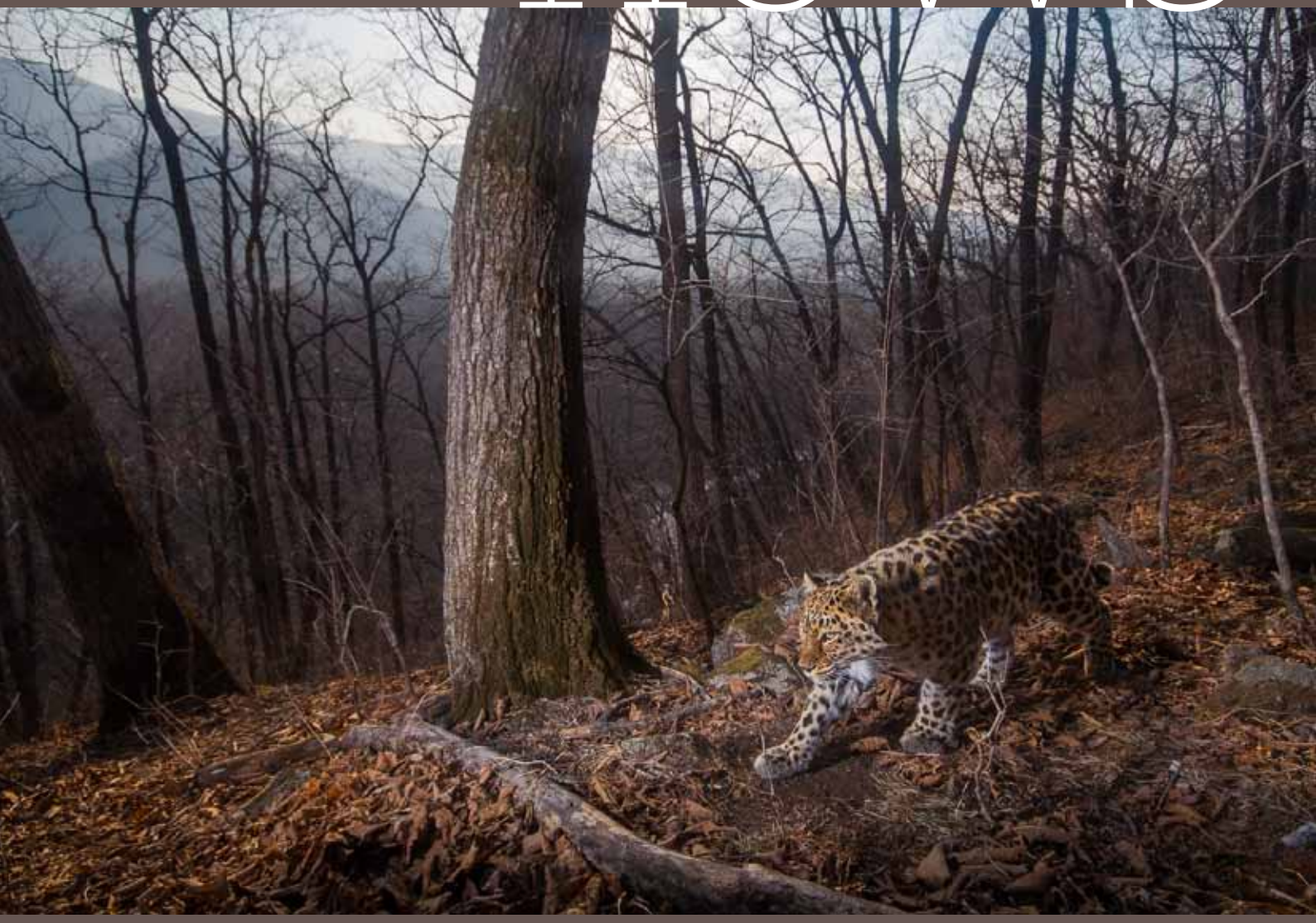

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Two Amur leopards come into captivity in Russia in 2015

Amur, or Far Eastern leopards *Panthera pardus orientalis*, rarely come into captivity, but in 2015, two animals were brought to the TRNGO Rehabilitation Centre in the Russian Far East. One animal was confirmed to have canine distemper, the first such documentation for this subspecies in the wild, and was eventually euthanised. Analyses of archived serum samples revealed there have been earlier exposures of the population to canine distemper. A male leopard brought into captivity was compromised by the loss of three toes, but with veterinary care he recovered, developed excellent hunting skills and seemed like a candidate for release back to the wild. However, icy conditions resulted in complications to the wounded paw, and a decision was made to retain him in captivity as part of the EAZA Amur leopard breeding programme.

Amur, or Far Eastern leopards are considered one of the world's rarest large felid subspecies. The lone remaining population of less than 100 Amur leopards occurs primarily in the forested areas of southwest Primorye, Russia (Hebblewhite et al. 2010) but is expanding into adjacent habitat in eastern Jilin Province, China (Feng et al. 2017). Land of the Leopard National Park LLNP, created in 2012, protects some 2,799 km^2 of Russian habitat for leopards and Amur tigers *Panthera tigris altaica*.

Located between Southwest Primorye and the city of Vladivostok, the TRNGO Tiger Rehabilitation Centre was built in 2012 to provide treatment and rehabilitation primarily for diseased and wounded tigers, as well as abandoned cubs. But it was also assumed that it would play a role, as needed, in treatment and rehabilitation of leopards. While tigers have been nearly continuously present in the rehabilitation centre since its opening, until spring of 2015 no needs arose for leopards. We report on the fate of two Amur leopards that were encountered in the wild and brought to TRNGO Centre in 2015.

Female Amur Leopard

On the evening of 08 May 2015, a female leopard estimated to be 22-24 months old was observed behaving abnormally along a gravel road in Khasanskii District (42°57'19.404'' N / 131°21'5.4'' E) inside LLNP. When staff from LLNP and the Wildlife Conservation Society WCS approached, the leopard showed a lack of fear, an indifference to its surroundings, and lacked coordination. The animal was remotely immobilised, moved to the TRNGO Centre, and placed in quarantine.

A preliminary veterinary examination revealed that the leopard was dehydrated and emaciated: she weighed 28 kg, compared to the 34 kg average weight of adult Amur leopard females (Miquelle unpubl. data). Analyses of serum, which was obtained from blood samples collected on 8, 11, 16 and 18 May were conducted at the A.N. Servertsov Institute of Ecology and Evolution in Moscow by S. V. Naidenko. Concentrations of immunoglobulin G to canine distemper virus CDV and to feline panleukopenia virus were measured using ELISA and Immunocomb kits. Antibody levels were below detection limits for both pathogens in samples collected on 8 May, but were detected in all subsequent samples, with a progressive increase in titers against CDV suggesting active infection. Further tests to confirm infection with feline panleukopenia virus were unavailable, but if present, it was likely subclinical due to the lack of gastrointestinal signs.

Efforts to support this female leopard had no noticeable effect and her condition continued to deteriorate (Fig. 1). On the 16th day of her stay at the facility (25 May 2015) it was decided to euthanise the leopard. A necropsy was performed and biological samples were taken for further examinations. The initial diagnosis of canine distemper was supported with comprehensive testing, including PCR amplification of CDV RNA based on the protocols used by Seimon et al. (2013), and histological examination of cerebral tissue (Sulikhan et al. 2018). Photos taken of this animal were compared to the comprehensive database of leopards from LLNP, but no match was found, supporting the supposition that this was a young individual that had not yet been captured by the extensive network of camera traps.

This case represented the first documentation of canine distemper in wild Amur leopards. To assess exposure history of the wild Amur leopard population, archived serum samples collected by WCS between 1993 and 2008 from 10 individuals were analysed. Antibodies were detected in two female leopards sampled in 1993 and 1994 (Sulikhan et al. 2018). However, unlike the female observed in 2015, which would have succumbed to complications associated with the disease if she were not euthanised, in both earlier cases animals were radiocollared and survived for at least 14 and 38 months, respectively, in the wild after sampling. Hence, while canine distemper can have serious impacts on wild felid populations (Roelke-Parker et al. 1996), and small isolated populations like that of the Amur leopard are most susceptible, we now know that this population has already withstood previous challenges from this disease.

Since CDV is unlikely to be sustained solely within a population of solitary felids (Gilbert et al. 2015), work is continuing to identify the likely source populations. Although dogs are a likely culprit, earlier work by Gilbert (2016) in this region suggested that small carnivores were likely responsible for maintaining the virus in the landscape. Although there has been no further evidence of a canine distemper "outbreak" in Southwest Primorye, this event is yet another reminder of such threats to such small, isolated populations, and a stimulus to move forward with plans to reintroduce a second population of leopards in Russia (Miquelle et al. 2010) as insurance against potentially catastrophic stochastic events.

Fig. 1. Female leopard, day after capture appeared disoriented and showed almost no reaction to the presence of people (Photo D. Miquelle).

Fig. 2. X-rays taken in March 2016, showing loss of phalanxes on three of the toes on the right front paw of male leopard Leo80M.

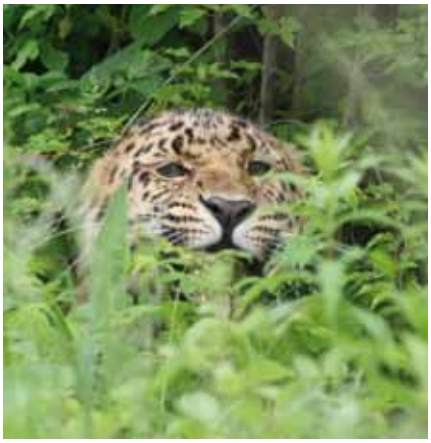

Fig. 3. Male leopard Leo80M showed aggression towards humans prior to negative conditioning (Photo TRNGO Rehabilitation Centre).

Fig. 4. Leo80M demonstrating his ability to climb trees within the enclosure despite the loss of three phalanges on his right front paw (Photo TRNGO Rehabilitation Centre).

Amur leopard male Leo80M

The second instance of an Amur leopard removed from the wild in 2015 began on 4 June in LLNP near the Chinese border. Border guards reported observing a leopard behaving abnormally. The animal was remotely immobilised (using a Dan-Inject gun and 5 mg/kg Zoletil® Virbac (tiletamine and zolazepam)) and delivered to the quarantine unit of the TRNGO Rehabilitation Centre.

Initial veterinary examinations indicated extreme emaciation and dehydration were likely the result of an injury to the front right paw, all contributing to the "abnormal behaviour" initially reported. Three toes were missing from this foot: three phalanxes were missing on the first toe, two phalanxes from the second and the first phalanx was missing on the third toe (Fig. 2). There was additional trauma to the soft tissues on these toes with uneven edges to the wounds. These injuries were consistent with trauma from a steel trap or possibly a snare. The leopard's weight at the time of arrival at the Rehabilitation Centre was 28.8 kg, much lower than the average weight of adult males (55 kg – Miquelle unpublisehd data). It had lost its appetite and was considered in critical condition. Fluids (Ringer's solution, 400 ml) were administered on the first day of treatment along with (5 mg/kg) of the antibiotic Enrofloxacin, which continued to be administered through the first week with a jab stick as needed. Convulsions on the second day were stopped with an intramuscular injection of 0.04 mg\kg Midazolam. The leopard's appetite returned to normal on the fifth day. During the first seven days the antibiotic Clindamycin was administered (10 mg\kg) by embedding capsules in meat. On the sixth day after capture, when the animal's condition was considered stable, he was immobilised a second time and surgery on the foot was performed to remove loose tissue, thoroughly clean and seal wounds on the toes. Laboratory tests of serum failed to reveal exposure to any infectious diseases, including canine distemper.

Comparing spot patterns using program Extract/Compare, the first photograph of this leopard was found in the camera trap database from nearby Jilin Province, China on 24 September 2014 when he was still following his mother. Based on photos, size, and tooth wear, this young male was estimated to be at least 2 years old.

Leo80M was held in quarantine for 34 days. During the first 10 days his diet consisted of small pieces of cut meat mixed with supplemental vitamins. Large chunks of domestic cattle (skinless) were gradually added to its diet.

On the 34th day in captivity, when wounds to its foot appeared fully healed, the leopard was transferred to an outdoor facility that included two linked enclosures totalling 1.7 ha. The total height of the fence was 5 m, with the top 1.5 m covered with sheet metal to prevent escape. At the top of the fence an inward 0.5 m overhang of fencing at 60° was added to further reduce chance of escape.

Leo80M was retained under observation at TRNGO Rehabilitation Centre for 375 days. Observations totalling 320 hours were performed using remote video cameras and trail cameras in video mode. Since this animal appeared to be a potential candidate for release back into the wild, we considered three factors in a decision-making process: 1) its ability to hunt natural prey, given injuries to its paw; 2) its response to humans, given the long time in an enclosure; and, 3) its overall health and status of the paw that had been damaged.

Hunting ability

To test this animal's ability to hunt we initially released domestic rabbits into his enclosure, gradually increasing the size of prey to sika deer (of different sex and age classes), and young wild boar. A total of 30 rabbits, 24 sika deer, and 3 wild boars were released into his enclosure during Leo80M's stay at TRNGO Rehabilitation Centre. We noted improvements in hunting skills over the first 12-14 released ungulates, as indicated by development of a clear sequence and complete suite of hunting behaviours (Yachmennikova et al. 2015). Over this time the leopard shifted tactics from simply chasing its prey with numerous unsuccessful attacks to concealment using features of the terrain in the enclosure. Observations suggested that by the 15th hunting sequence on ungulates this animal appeared highly skilled and experienced. It was not clear if the improvements were associated with normal developmental skills of a young leopard, or adaptations and adjustments to hunting with a wounded foot. If the animal were just two years, as estimated, it was likely still in its first few months of solitary existence, and perhaps had not yet developed proficient hunting skills. In either case, by day 184 we felt that this animal demonstrated hunting skills sufficient to survive in the wild.

Attitude towards humans

During his first five days in captivity Leo80M was in critical condition and showed no

interest in his environment or humans. Reactions to humans and external stimuli reappeared on the 5th and 6th days. As his condition improved he became more aggressive towards humans. Once released into an open enclosure, he became more wary of humans. While still being fed meat, the leopard kept a distance of at least 5-10 m from the keeper. After his diet was changed to live prey only, the distance to keeper increased to 20-25 m. We also tested his reactions to humans by having an individual unknown to Leo80M approach the enclosure. When being fed meat, the leopard maintained a distance of 20-25 m from the unknown individual. This distance increased to 25-35 m when his diet shifted to natural prey that he hunted. However, in 4 of 16 cases the leopard still retained visual contact with the human (Fig. 3), and in one instance he charged the person. To try and correct this behaviour, we used a hand flare and a dart (without a needle) fired from a dart gun when the animal remained in close proximity to a human. We conducted this negative conditioning three times, after which the leopard avoided visual contact with humans.

3. Healing of paw

Upon release into the enclosure, we saw no obvious effects of the injury to the paw (no limping). During the rehabilitation process its success in killing large prey suggested his injuries did not limit his ability to successfully hunt. He also regularly climbed trees (essential behaviour to avoid predation by tigers in the wild; Fig. 4). However, as snow fell and crusty ice developed in late February and early March, the leopard began limping again, and while sitting, often held the wounded paw up off the ground. Leo80M was immobilised and upon examination, we noted chronic wounds on the paw with acute inflammation where the skin was in constant contact with the ground. These developments may have been related solely to the icy ground substrate, which was temporary, but since snow and ice are common in southwest Primorye, where he would be released, it was determined that this animal was not a suitable candidate for release. In preparation for life in captivity, his diet was changed to processed meat, and the process of acclimation to humans began.

While disappointing, our work provides useful experience for future rehabilitation of wild leopards or, if necessary, the preparation of captive leopards for release into the wild. While it was not possible to confirm the cause of the injury, wounds were consistent with those derived from a steel jaw trap or a wire snare. Use of steel jaw traps is illegal in Southwest Primorye and are virtually non-existent in nearby China, but wire snares are commonly encountered there (though illegal). Although deaths of tigers and leopards in wire snares have been reported in northeast China, it was impossible to confirm the source of this trauma.

On 14 June 2016 LeoM80 was captured and transferred to the Moscow Zoo's Center for Reproduction of Rare Species on the outskirts of Moscow, where it will be introduced into the EAZA Amur leopard breeding programme. Over the past forty-five years only two other animals from the wild have been introduced into the European Amur leopard captive breeding programme and therefore Leo80M represents an important genetic addition to the captive population. We are hopeful that his offspring may one day return to the wild as part of the planned Amur leopard reintroduction programme (Miquelle et al. 2012).

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