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The Blue Dyeing Poison-Dart Frog, *Dendrobates tinctorius* (*Dendrobates azureus*, Hoogmoed 1969): extant in Suriname based on a rapid survey

[1,*]Christian A. d'Orgeix, [1]De'Jah Hardy, [1]Sarah Melissa Witiak, [2]Laren R. Robinson, and [3]Rawien Jairam

[1]*Department of Biology, Virginia State University, Petersburg, Virginia, 23806, USA* [2]*Department of Agriculture and Ecology, Virginia State University, Petersburg, Virginia 23806, USA* [3]*National Zoological Collection of Suriname, Anton de Kom Universiteit, Paramaribo, SURINAME*

***Abstract.*—The blue color morph of *Dendrobates tinctorius*, originally described as *D. azureus*, has only been reported from a few forest islands surrounded by the Sipaliwini savanna in Suriname, South America. The last published survey of these populations occurred in 1996. The threats of emergent diseases, illegal collecting, climate change, and habitat destruction through anthropogenic fires toward these populations are unknown. This report presents the results of a rapid survey of the three forest islands where *D. tinctorius* historically occurred to assess its current status. One 50 x 50 m survey plot was established in each of these three forest islands. A total of 23 frogs were recorded, with some individuals found in each of the forest island surveyed. These results indicate that *D. tinctorius* populations are still present at all three historical localities surveyed by previous researchers in 1968 and again in 1996, although the current surveys found fewer frogs. During the surveys there was no evidence of illegal collecting or habitat degradation. These observations provide baseline data that can be used for future monitoring and protection of one of the most geographically restricted and unique color morphs of *D. tinctorius*.**

Keywords. Amphibia, Dendrobatidae, conservation, Sipaliwini savanna, population decline, habitat fragmentation, climate change

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Introduction

The Blue Dyeing Poison-dart Frog was described a half-century ago as *Dendrobates azureus* (Hoogmoed, 1969) and subsequently synonymized with *D. tinctorius* (Wollenberg et al. 2006). The inclusion of this species as a color morph of *D. tinctorius* was also confirmed by Noonan and Gaucher (2006). Ouboter and Jairam (2012) considered this species to be a subspecies of *D. tinctorius* due to the geographically isolated occurrence of this particular morph. Its classification as a subspecies was refuted by Frost (2017) who maintained this species as *D. tinctorius*. Other blue morphs of *D. tinctorius* were documented by Silverstone (1975) from Shudikar-wau, Guyana and by Avila-Pires et al. (2010) from Estaçáo Ecológica Grão-Pará North, Brazil. However, specimens from Suriname do not display the typical light *D. tinctorius* pattern on the dorsum, whereas specimens from the other mentioned locations still have this pattern.

The predominantly blue color morph ("azureus") found in Suriname is restricted to a few isolated "forest islands" in the Sipaliwini savanna in southern Suriname (Hoogmoed 1969; Riezebos 1979; Cover 1997; Ouboter and Jairam 2012). This area was mapped in July 1935 when an expedition by Baron van Lynden was charged with establishing the southern border of Suriname (Lynden 1939). The Sipaliwini savanna is part of a larger savanna complex covering portions of Suriname and northern Pará, Brazil (Paru savanna). The current vegetation primarily consists of grasses, sedges, scattered shrubs, trees, and *Mauritia* palms (Oldenburger et al. 1973). The sharp demarcation between the savanna and forest islands may be due to geological factors as suggested by Hoogmoed (1973), while the anthropogenic influence of fires set during the dry season by the indigenous people might have been a reason that the savanna remained open (Oldenburger et al. 1973; Ouboter and Jairam 2012) [Fig. 1].

A number of factors potentially threaten the continued existence of these populations of *D. tinctorious* in the wild. Their extremely restricted range and small population size are factors that increase the risk of extinction (Hall et al. 2008). Anthropogenic fires could potentially reduce

Correspondence. *cdorgeix@vsu.edu

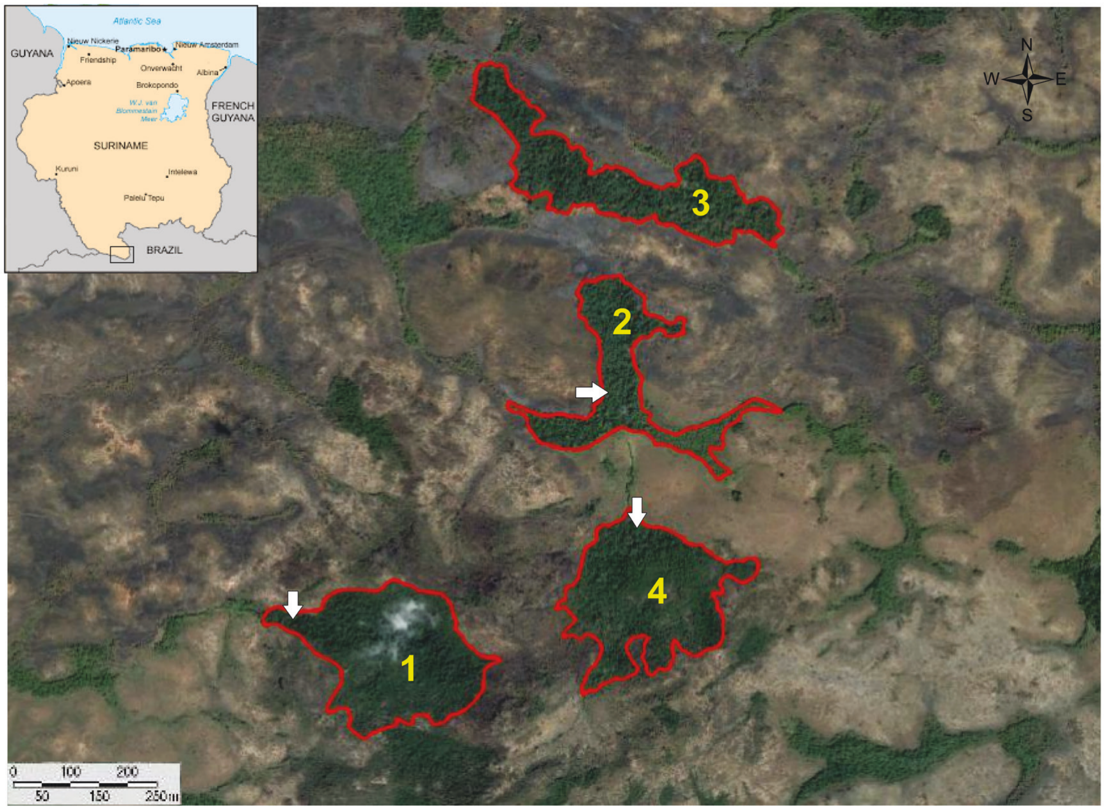

Fig. 1. Map of Suriname, South America (upper left inset) with approximate location of Sipaliwini savannah indicated by the black box. Forest Islands are outlined in red and identified by numbers corresponding to those of Hoogmoed (1969, 2019). Arrows indicate locations of the 50 x 50 m plots surveyed in Forest Islands 1, 2, and 4 during 16–18 June 2015. Forest Island 3 was not surveyed. In this 2004 Google image, savanna vegetation surrounding the forest islands had been recently burned by indigenous hunters.

the size of the forest islands, and charred trees on the periphery of the forest islands were noted by Cover (1997). The emerging infectious disease *Batrachochytrium dendrobatidis* (*Bd*) may put these populations at risk (Courtois et al. 2012, 2015; James et al. 2015). Although *Bd* has not been documented in Suriname, it has been documented in *D. tinctorius* in French Guiana (Courtois et al. 2015) which is east of Suriname. In addition, the blue color morph of *D. tinctorius* occurring in the Sipaliwini savanna is potentially vulnerable to rarity-fueled exploitation for the pet trade (Hall et al. 2008).

Despite having been discovered almost 50 years ago (Hoogmoed 1969), the only reported subsequent attempt to ascertain the status of the blue morph of *D. tinctorius* in the Sipaliwini savanna occurred in 1996 (Cover 1997). At that time Cover (1997) found frogs but reported potential evidence of illegal collection. Thus, the primary objective of the current study was to determine the current status (presence or absence) of *D. tinctorius*, in the Sipaliwini savanna. If present, the plan was to establish survey plots and conduct preliminary surveys to serve as benchmarks for future population studies.

Materials and Methods

Survey plots. A survey plot measuring 50 x 50 m was established in each of three forest islands which were separated by approximately 350–750 m of tall grass savanna. Forest islands were identified using the same numbering scheme as Hoogmoed (pers. comm.) [Fig. 1]. Survey plots within the forest islands measured approximately 4% of 6 ha (Forest Island 1), 12.5% of 2 ha (Forest Island 2), and 6% of 4 ha (Forest Island 4) [Fig. 1]. Forest Island 3, which lies ~300 m north of Forest Island 2, was not surveyed due to time constraints. Plot placements were determined by entering each of the forest islands and visually searching until a *D. tinctorius* was encountered (Fig. 2). The nearest stream was then used as one side of the 50 x 50 m sample plot, based on previous research by Hoogmoed (1969) and Cover (1997) citing stream preference by the frogs. Plot corners were marked with GPS coordinates using a Garmin 60CSx. All three forest islands were subsequently digitized using images from Google Earth Pro, which were further edited in ArcMap, version 10.2, to show the locations where the surveys were conducted. To do this, Google Earth imagery was acquired and added as a layer into ArcMap. Then Arc toolbox's KML layer feature was used to transfer the digitized forest islands created in Google Earth to ArcMap. The resulting image emphasizes the presumed isolation by habitat of the *D. tinctorius* populations from one another (Fig. 1).

Survey plots 1 and 4 were bordered on three sides by small clear water streams. Survey plot 2 was characterized

Fig. 2. *Dendrobates tinctorius* from Sipaliwini savanna, Suriname.

Fig. 3. Stream and boulder habitat in Survey Plot 1, Sipaliwini savanna, Suriname.

by having a small clear water stream at the lowest part of the plot. Survey plot 1 was located on a north-facing slope that rose from 315–340 m in elevation. The area was under a fully closed canopy forest with dense understory vegetation interspersed with lianas. The forest floor was covered by leaf litter, decomposing fallen trees, and boulders of about 2–3 m in height and 3–6 m in diameter (Fig. 3). Survey plot 2 was on a southeast facing slope that rose from 315–325 m in elevation. The plot was under a closed canopy with an open understory and a forest floor covered by leaf litter. There were no boulders in this plot. Survey plot 4 was on a west-facing slope that rose from 300–350 m in elevation. The area was under a fully closed canopy with dense understory vegetation. One-third of the plot on the southwest side had a dense understory of *Phenacospermum* sp. and *Poaceae* bamboo species. The forest floor was covered by leaf litter interspersed with 10–15 clumped boulders that were about 1–3 m high and 1–5 m in diameter.

Survey methodology. Each plot was visually surveyed once, for varying amounts of time, from 1030–1300 h. Surveys occurred on 16–18 June 2015, three days after the last rainfall on 13 June 2015. To survey a plot, nine individuals were spaced approximately 1.5 m alongside each other forming a line. Starting at one corner of the plot, the surveyors walked 50 m to the opposite side of the plot. Upon reaching the far side the surveyors again spaced laterally starting 1.5 m from the person on the farthest end to cover the next section, and walked back towards the opposite side. This process was repeated until the whole plot was surveyed. Survey participants wore sterile disposable gloves which were changed between the captures of each individual. Capture locations of individual frogs were recorded as GPS coordinates using a Garmin 60CSx. Frogs were then placed in individually labeled Zip-Lock bags. Snout-vent length (SVL) was measured to nearest 0.1 mm with digital calipers. Adults were not sexed. After measurements were taken, each frog was released at its original point of capture.

To identify potential changes in perimeter sizes of the forest islands due to environmental or anthropogenic factors, the most historical and most recent available Google Earth images from 31 December 1969 and 17 November 2004 were used. These images were visually compared by juxtaposing the forest islands outlined in red, from 2004 (Fig. 4A) onto the 1969 image (Fig. 4B).

Statistical methods. A General linear model ANOVA was used to compare SVL values between specimens from each forest island. A *t*-test was used to compare SVL of frogs measured in the current survey with those reported by Hoogmoed (1969: Table I). SVL are reported to nearest 0.1 mm ± 1 SD. All analyses were conducted using Minitab 17 statistical software (Minitab Inc., State College, Pennsylvania, USA).

Results

Twenty-two adults and one juvenile were found in the three forest island plots surveyed. Sixteen frogs were found during the surveys, and seven were found adjacent to the survey plots. Two frogs were found in Forest Island 1 in 1 h (270 person-min/frog). Four frogs were found adjacent to the survey plot in Forest Island 1 in 0.5 h (67.5 person-min/frog). Four frogs were found in Forest Island 2 in 0.5 h (67.5 person-min/frog). Ten frogs were found during the survey of Forest Island 4 in 1.5 h (81 person-min/frog). Three frogs were found adjacent to the survey plot in Forest Island 4 in 0.5 h (90 person-min/frog). The overall mean yield of encounters was 115.2 person-min/frog. Variations in time spent conducting a survey often reflected the difficulty in traversing the plot based on the physical obstructions encountered (e.g., boulders and density of herbaceous undergrowth).

Adult SVL values ranged from 36.7–46.4 mm, with a mean of 42.0 ± 3.1 mm. No significant differences between SVL of adult frogs from different forest islands were found (one-way ANOVA: $F_{2,21} = 0.23$, $P = 0.795$). No significant difference was found in a comparison of SVL values recorded by Hoogmoed (1969) and the current survey (*t*-test: $t = 1.49$, $df = 44$, $P = 0.144$). The single juvenile recorded here measured 18.9 mm SVL.

A comparison of the vegetative perimeters of Forest

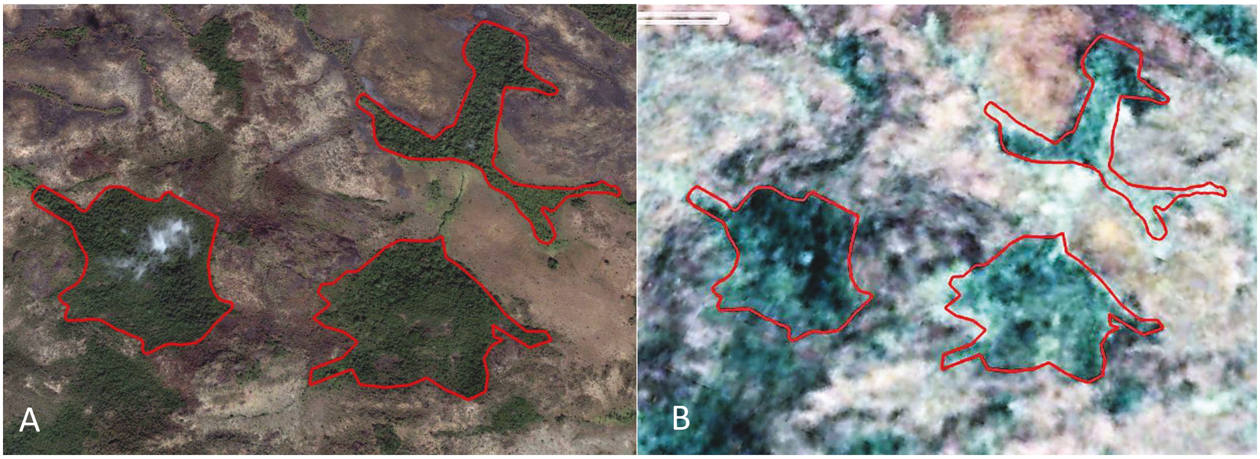

Fig. 4. Forest Islands 1, 2, and 4, extant vegetation comparison between Google Earth images for 2004 (A) and 1969 (B). Red outlines delineating the three forest islands were juxtaposed from the 2004 image onto the 1969 image.

Islands 1, 2, and 4 appear almost identical in the two Google Earth images taken in 2004 (Fig. 4A) and 1969 (Fig. 4B).

Discussion

Survey findings. The survey sites represented three of the four forest islands where frogs were found in the surveys by Hoogmoed (1969) and Cover (1997). In the current surveys only adults were found, with the exception of a single juvenile. No males transporting tadpoles were found. Hoogmoed (1969) reported a male carrying two tadpoles on his back on 30 September 1968, while Cover (1997) who surveyed in June, did not note this behavior, although he reported newly metamorphosed individuals and tadpoles at two sites.

This survey recorded fewer frogs (23) than either Hoogmoed (1969) or Cover (1997). Cover (1997, pers. comm.) reported 56 frogs from Forest Island sites 1, 2, and 4. Hoogmoed (1969, 2019) reported 82 frogs from Forest Islands 1–4, during 11 different dates in 1968 and 1970. Differences in methodologies, season, and weather limit most direct comparisons between these studies. Both Cover (pers. comm.) and Hoogmoed (1969, 2019) concentrated their searches in suitable habitat along stream courses, while in the current survey the 50 x 50 m plots were centered around the spot where a frog was first encountered. Weather events, such as rainfall, presumably influence frog activity. For example, Cover noted that after heavy rains at one site, the numbers of frogs visible on the forest floor increased dramatically (J. Cover, pers. comm.). A similar situation was also reported by Wevers (2007). The current surveys took place 3–5 days after the last rains. Hoogmoed (1969, 2019) reported 82 frogs during herpetological inventories conducted on 11 different days in September and October 1968 and February 1970. In comparing the time required to encounter a frog, Hoogmoed (2019) averaged 10.1 person-min/frog while the average in the current survey was 115.2 person-min/frog. This would suggest a significant population decline if all other factors were equal.

The blue morph of *D. tinctorius* in the Sipaliwini savanna forest islands appears to be both geographically and genetically isolated from other color morphs of *D. tinctorius* in Suriname. The nearest known populations of *D. tinctorius*, which are black and yellow dorsally, are approximately 23 km north of the closest forest islands surveyed (Fouquet et al. 2015). Blue morphs of *D. tinctorius* in Sipaliwini are more than 300 km away from the blue morphs reported by Silverstone (1975), and approximately 315 km from the blue morphs reported by Avila-Pires et al. (2010). In addition, the frogs in each of the forest islands surveyed may be genetically isolated from each other. Exploration of other remote forest islands in the Sipaliwini savanna region may reveal additional isolated populations of blue *D. tinctorius.* Future molecular analyses could elucidate the genetic divergence in these forest island populations since their presumed isolation approximately 10,000 years ago (Riezebos 1979).

Conservation considerations and future threats. *Batrachochytrium dendrobatidis* (*Bd*) has been recorded in French Guiana (Courtois et al. 2012, 2015) and Brazil (Becker et al. 2016) which border Suriname to the east and south, respectively. During this rapid survey no dead frogs or frogs displaying visual symptoms of *Bd* were found, although seemingly healthy specimens are still known to be carriers of this disease (Coutinho et al. 2015).

While no evidence was found of illegal collecting for the pet trade, that lack of evidence is not proof that it does not occur or will not occur in the future. The indigenous Trio peoples control both access to the transportation to the savanna (canoes) and permission to visit the forest islands where the frogs are found. Trio culture recognizes the uniqueness of these frogs. Permission from the village chief must be granted to visit the sites where frogs may be observed, but collection is not allowed. This is enforced by the accompanying Trio guides. Presently, the Trio people's stewardship practices provide a large measure of protection from collecting for the pet trade. Sustainable programs that benefit the Trio community should be implemented to reinforce their stewardship of the habitat and wild populations of this unique color morph.

Visual comparison of the perimeter size of these forest islands between the 2004 and 1969 images do not appear to indicate a significant change in size, despite the history of anthropogenic fires (Fig. 4A,B). Cover (1997) reported charred wood on the periphery of the forest islands. However, when he observed a fire in the savanna, it stopped when it reached the lush vegetation on the perimeter of a forest island (J. Cover, pers. comm.). Although the forest islands appear to be stable in size, due to their small area and isolation, any reduction in the water sources either through drought or climate change could threaten both the forest island vegetation and associated stream habitat. An increase in xeric conditions could increase the risk of forest island vegetation becoming drier on the periphery and thus more susceptible to anthropogenic fires resulting in the edges of these islands moving inwards, leading to a decrease in their size or even complete destruction.

Barring direct human collection, climate change may be the most serious threat to this unique blue morph of *D. tinctorius.* There is a growing body of evidence that tropical species will need to undergo elevational or latitudinal range shifts to remain in analogous climatic conditions in the future (Colwell et al. 2008; Nowakowski et al. 2016). Given the isolated nature of these forest islands, their lack of connectivity with either cooler forests or altitudinal refugia, any change to the forest island habitat may be the biggest threat to the continued survival of this unique *D. tinctorius* blue morph in the wild.

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Christian d'Orgeix is a behavioral ecologist and conservation biologist at Virginia State University. Christian's research focuses on the mating systems and conservation biology of reptiles and amphibians. One of his current projects examines the probability of extinction of high- and low-elevation populations of lizards. In Suriname, Christian and his students collaborate with Dr. Paul Ouboter and Rawien Jairam from Anton de Kom Universiteit, Suriname, on studies on the behavior and conservation of frogs and lizards.

Rawien Jairam works at the National Zoological Collection of Suriname and is co-author of the book *Amphibians of Suriname.* Rawien has an M.Sc. in Conservation Biology and has been interested in the herpetofauna of Suriname for many years. Apart from general herpetology, he is specifically interested in taxonomy and species distributions.

De'Jah T. Hardy is a junior Biology major at Virginia State University. De'Jah traveled to Suriname, South America, to conduct research on frogs and ants. She also traveled to China to take Clinical Medicine classes at Jinan University in Guangzhou. De'Jah's goal is to explore as many opportunities as she can as an undergraduate, and then go straight for her Master's in Psychology. She plans to travel the world someday, helping children as a traveling Occupational Therapist.

Sarah Melissa Witiak is a plant biologist who received her Ph.D. from Pennsylvania State University (University Park, Pennsylvania, USA) and currently teaches at Virginia State University. Sarah is primarily interested in the "pretty" parts of biology, including poison-arrow frogs, insect galls, and flower evolution and ecology. Her current projects include studies of plant volatiles and a survey of insect gall diversity in Suriname.

Laren Robinson worked as a GIS specialist at Virginia State University. She is currently pursuing career options in GIS applications. (No photo available)