

1 **Conserving Panamanian Harlequin Frogs by Integrating Captive-breeding and Research**
2 **Programs.**

3
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19 **Highlights**

- 20 • *Atelopus* species have experienced severe chytridiomycosis-related declines.
- 21 • This case study documents the wild and captive status of *Atelopus* in Panama.
- 22 • Habitat models improve historical distribution maps and guide future efforts.
- 23 • Captive breeding efforts prevent extinctions and are a valuable research resource.

24
25 **ABSTRACT**

26 Captive breeding programs are a valuable conservation resource especially when integrated with
27 research goals. Panamanian Harlequin frogs (genus *Atelopus*) serve as a case study for
28 integrating captive breeding and research goals because they have experienced drastic
29 chytridiomycosis-related declines and have large captive populations. We reevaluated all
30 Panamanian *Atelopus* species through the IUCN Redlist and compiled occurrence records for
31 Panamanian *Atelopus* species to create improved historical distribution maps. We model
32 *Atelopus* habitat suitability to improve our knowledge of their likely range and to guide for future
33 conservation and reintroduction efforts. Captive breeding efforts in Panama and the United
34 States established secure *ex-situ* populations of *Atelopus certus*, *A. glyphus*, *A. limosus*, *A. varius*,
35 and *A. zeteki*. *Atelopus chiriquiensis* is presumed to be extinct with no captive populations. The
36 status of one undescribed species, *Atelopus* aff. *limosus*, has not been evaluated and no secure
37 captive population has yet been established. Captive breeding efforts that produce a surplus of
38 *Atelopus* are an important resource for disease mitigation research and have enabled release trials
39 to begin adaptive management approaches to understand the factors limiting *Atelopus*
40 reintroduction efforts. The recent proliferation of molecular tools, climate models, bio-banking,
41 and reproductive technologies position us to address multiple applied and basic evolutionary
42 questions such as: What factors cause differential disease outcomes? Do persisting populations
43 have heritable traits associated with improved survivorship? Are there climatic refugia from
44 disease? Ultimately, the answers to these questions will help us develop applied solutions and
45 facilitate reestablishment of self-sustaining wild populations.

47 Keywords: *Atelopus*, captive-breeding, conservation, chytridiomycosis, amphibian.

48

49 **Introduction**

50 Amphibian populations are declining globally with more than one third of evaluated species
51 being listed as globally threatened (Stuart et al. 2004). The disease chytridiomycosis, caused by
52 the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*) is one major threat that is decimating
53 amphibian populations worldwide (Berger et al. 2016). With no current tools to mitigate this
54 threat, ex situ conservation programs have been set up to prevent imminent extinctions with the
55 hope that species will eventually be reintroduced to the wild (Zippel et al. 2011).

56

57 Harlequin frogs in the genus *Atelopus* are among the most threatened amphibians in the world
58 (Lötters 2007). Threats to *Atelopus* include habitat modification and collection for the pet trade,
59 but *Bd* is the most pressing threat, responsible for catastrophic declines and disappearances
60 throughout their range (La Marca et al. 2005; Berger et al. 2016). Panama has six described
61 species of *Atelopus*: *A. certus*, *A. chiriquiensis*, *A. glyphus*, *A. limosus*, *A. varius*, *A. zeteki*, and at
62 least one undescribed species: *Atelopus* aff. *limosus* (Flechas et al. 2017). All of the described
63 species are listed as Critically Endangered or Extinct by the IUCN (IUCN SSC Amphibian
64 Specialist Group *In press*). There is currently no solution for *Bd* likely to prevent the continued
65 decline and extinction of *Atelopus* species in Panama, making ex situ captive assurance colonies
66 one of the only options for their conservation (Gratwicke et al. 2016).

67

68 Captive breeding is expensive and requires a huge amount of effort, often with no clear end date,
69 making fundraising a demanding task subject to donor fatigue. As a result, breeding programs
70 are subject to the common criticism that funds should be prioritized elsewhere. However, the
71 benefits of captive breeding go beyond simply preventing a species' extinction. Robust captive
72 populations allow for research to improve collections management and sustainability, to
73 understand and mitigate disease, and to increase the chances for successful reintroduction.

74

75 This paper uses Panamanian *Atelopus* as a case study for captive breeding as a conservation
76 action. We summarize the conservation status of *Atelopus* species both in the wild and in
77 captivity. As part of the wild assessment we update known historical distribution maps and
78 model habitat suitability for *Atelopus* in Panama to inform future conservation and reintroduction
79 efforts. We discuss recent scientific advances using captive populations that move us towards
80 science-based conservation solutions.

81

82 **The Role of Captive Breeding Programs**

83 Captive breeding and reintroduction are two priorities of the Global Amphibian Conservation
84 Action Plan (Gascon et al. 2007). Of the more than 7,900 described amphibian species, 77 now
85 have active captive breeding and reintroduction programs (Harding et al. 2016). These programs
86 are often used to develop genetically viable, sustainable captive populations, as well as to grow
87 and maintain suitable infrastructure and capacity to support those activities (Griffiths and
88 Pavajeau 2008; Harding et al. 2016). Captive populations of animals also serve as living
89 ambassador animals with an incredible power to engage audiences, and education programs are a
90 critical element to captive breeding programs that help to build public support for the
91 conservation of these species (Zippel et al. 2011).

92 In Panama, the first efforts to establish assurance populations of amphibians in response to the
93 *Bd* threat began in 2001 when Panamanian golden frogs (*A. varius* and *A. zeteki*) were exported
94 to U.S. zoos to be managed as part of a species survival program (Zippel 2002). Later efforts
95 built the physical infrastructure and staffing capacity needed to house additional at-risk species
96 in Panama itself (Gratwicke and Murphy 2016). A prioritization exercise examining 214 species
97 of Panamanian amphibians found that *Atelopus* species were among the species at highest risk
98 for *Bd*-related extinctions, and they were expected to have the best chances of avoiding
99 extinctions through captive breeding efforts (Gratwicke et al. 2016).

100

101 The Panama Amphibian Rescue and Conservation Project (PARC) is based at the Smithsonian
102 Tropical Research Institute (STRI) in Panama and holds captive assurance populations of 12
103 amphibian species, including five *Atelopus* species (Box 1). The rapidly growing captive
104 *Atelopus* populations in Panama have exceeded most of the captive management goals (Fig. 1,
105 PARC 2017). Management options now include selective rearing of limited numbers of
106 offspring, euthanizing surplus-bred juveniles, or allocating surplus frogs for research. STRI hosts
107 1,400 visiting scientists, students and interns per year at 6 research stations around the country.
108 This infrastructure and the ability to host visiting researchers uniquely positions the PARC
109 project to integrate captive breeding and research goals as access to *Bd*-susceptible animals is a
110 critical resource for testing conservation-relevant hypotheses.

111

112 *Research to improve collections management and sustainability*

113 Many research priorities associated with captive collections are designed to help solve problems
114 that affect the sustainability or improve cost-effectiveness of captive rearing. For example,
115 research on stress hormones helped to establish cost-effective group housing recommendations
116 for *Atelopus* (Cikanek et al. 2014), while research on the causes of spindly leg syndrome
117 generated new solutions for treatment of this lethal condition associated with captive populations
118 (Camperio et al. 2018). Collection and treatment of large numbers of *Bd*-positive *Atelopus* from
119 the wild as they were brought into captivity offered veterinarians a valuable chance to optimize
120 disease screening and treatment protocols using a case-study approach (Baitchman and Pessier
121 2013).

122 Hormone dosing methods can now be used to help improve representation of difficult-to-breed
123 founding animals and collect gametes to build cryopreserved genome resource that can safeguard
124 against unintended genetic bottlenecks in captivity (Kouba and Vance 2009). Cryopreservation
125 methods for amphibians have not been perfected, but live amphibian offspring have been created
126 from cryopreserved spermatozoa (Clulow and Clulow 2016), and comprehensive biobanking
127 protocols have been developed for Panamanian species. Even though we have not yet produced
128 live offspring from frozen *Atelopus* sperm, work has begun on cryopreserving tissue and sperm
129 for all Panamanian *Atelopus* (Della Togna et al. 2017). In the future, assisted reproduction
130 technology could be applied to collect sperm from relict populations that may have survived the
131 *Bd*-outbreak and introduce genotypes that are resistant or tolerant to *Bd* into the captive
132 populations. Regardless, genome resource banks and tissue collections of rare species are an
133 invaluable resource for basic research as high-quality tissues are needed for genomic research
134 that are not normally available for species on the brink of extinction (Comizzoli and Wildt 2017).

135 *Research to understand and mitigate disease*

136 Most instances of natural *Bd* infections in wild *Atelopus* populations have been associated with
137 serious declines that ended in species non-detection (Table 1). As long as we have captive
138 populations of susceptible *Atelopus* species, the incentive remains to continue working on
139 finding a solution to the chytridiomycosis problem. We do not yet have a realistic solution to
140 manage *Bd* in nature (reviewed by Garner et al. 2016), but a significant body of knowledge on
141 the threat of *Bd* to *Atelopus* has been derived using surplus-bred captive animals. Captive
142 *Atelopus* populations helped us understand disease dynamics under different climatic scenarios
143 (Bustamante et al. 2010) and observe disease dynamics within multispecies community
144 assemblages (Di Renzo et al. 2018). They have also allowed research that characterized genomic
145 responses to infection (Ellison et al. 2014; Ellison et al. 2015), evaluated putative changes in *Bd*
146 virulence (Langhammer et al. 2013; Voyles et al. 2018), and assessed the effectiveness of
147 behavioral (Sauer et al. 2018) and innate defenses (Voyles et al. 2018) against *Bd*.
148

149 One initial *Bd* mitigation approach that has been investigated for *Atelopus* is augmentation of the
150 antifungal skin bacteria community to help prevent disease (also see Vredenburg et. al. this
151 volume). Despite concerted research into *Atelopus* skin microbiomes (Flechas et al. 2012; Becker
152 et al. 2014; Becker et al. 2015b; Rebollar et al. 2016), development of a probiotic disease
153 mitigation protocol has been thwarted by difficulties in achieving lasting manipulations of
154 *Atelopus* skin microbiomes (Becker et al. 2011; Becker et al. 2015a). While the findings of
155 *Atelopus* microbiome studies have led to intriguing patterns of associations between disease and
156 microbes (e.g. Becker et al. 2015b), difficulty controlling symbiotic microbial community
157 composition is a common situation hindering probiotic applications in all systems (Yong 2016).
158 It appears that we need more basic research to understand what influences microbial community
159 structure and function before we can jump to applied solutions.

160 The recent discovery that recovering populations of Panamanian amphibians may have evolved
161 more effective antifungal skin secretions (Voyles et al. 2018) offers the potential to translocate
162 resistant genotypes to sites where they do not currently exist, but where suitable habitat exists
163 (Mendelson et. al. this volume). Captive populations will be an invaluable asset to this type of
164 activity as breeding resistant or tolerant genotypes within an existing captive population
165 infrastructure can produce large numbers of frogs with minimal impacts to small source
166 populations that could be severely impacted by removal of a small number of individuals. It is
167 possible to collect and evaluate the effectiveness of anti-*Bd* skin secretions using a non-lethal
168 standardized assay (Woodhams et al. 2006). Using skin secretions as an indicator of
169 susceptibility could allow us to screen captive frogs and breed for resilience traits without
170 exposing the animals to a pathogen, solving at least some of the ethical issues that might arise
171 from large-scale assisted evolution trials involving disease exposure. Similar assisted evolution
172 approaches are being actively explored to breed strains of coral that are resilient to climate
173 change (Van Oppen et al 2017).

174 Another genomic approach is to identify genes associated with disease resistance or tolerance
175 (eg. Savage and Zamudio 2011) and to use marker-assisted breeding. Often, desirable traits are
176 associated with large numbers of genes that each have small effects on the phenotype, which is
177 why marker-assisted animal breeding has not been widely adopted. Technological advances now
178 allow us to associate thousands of single nucleotide polymorphisms with desirable traits for
179 genome selection, improving the prospects for wider adoption of this method (Meuwissen et al.

180 2016). However, recent transcriptome studies found that desirable disease outcomes in one
181 *Lithobates* species were associated with under (rather than over) expression of innate and
182 acquired immune genes (Savage et al. in revision), suggesting that marker-assisted breeding for
183 chytridiomycosis resistance may prove difficult.

184 Other potential approaches include hybridization with closely related resistant species as a form
185 of genetic rescue or through more direct genetic engineering, which are solutions that have both
186 been successfully applied to American chestnuts that were wiped out by the chestnut blight
187 (Steiner et al. 2017). CRISPR/Cas 9 methods for genome editing have been successfully applied
188 primarily to *Xenopus laevis* and *X. tropicalis* whose full genomes have been sequenced and are
189 publicly available (Shigeta et al. 2016). Targeted gene disruption experiments are rapidly
190 illuminating gene function in this model (Shigeta et al. 2016). As of 2018, the genomes of five
191 anuran species have been sequenced and published (Edwards et al. 2018). Improvements in
192 genome sequencing technology and assembly pipelines mean that many more large amphibian
193 genomes are likely to become available in the near future. With this progress it is conceivable
194 that our understanding of the genetic basis for *Bd* resistance will improve to the extent that we
195 might eventually be able to genetically engineer susceptible frogs to resist infection. While these
196 cutting-edge solutions are exciting, the advances they offer are built on a foundation of basic
197 research that involves established assisted breeding methods, reference genomes, differential
198 gene expression studies and research into skin peptides. All of these basic research efforts will be
199 greatly enhanced by integration with captive breeding efforts and access to genome resource
200 banks that are only just being established as part of the Global Amphibian Conservation Action
201 Plan (Gascon et al. 2007).

202 *Research into Historic Distribution and Reintroduction*

203 A goal of the Panama Amphibian Rescue and Conservation Project is reintroduction and
204 reestablishment of wild *Atelopus* populations in Panama. One required step for reintroduction
205 plans is understanding species' historic distributions and their habitat needs (IUCN 2013). We
206 used historic occurrence data and known habitat requirements to improve distribution maps for
207 all Panamanian *Atelopus* species (Fig 2A). We also used Maxent 3.4.1 to model habitat
208 suitability for *Atelopus* in Panama and Costa Rica. Maxent is a maximum entropy method for
209 modeling species' geographic distributions based on environmental factors that has been shown
210 to work well when only presence data is available (Phillips et al. 2006; Elith et al. 2006; Phillips
211 et al. 2017). See online appendix for details on the data and methods used for the Maxent model.

212
213 Our final Maxent model had good performance and predicted areas of habitat suitability were
214 reasonable based on our understanding of the ecology of Panamanian *Atelopus*. The mean Area
215 Under the Curve (AUC) was 0.685 (Fig. A1). AUC is often incorrectly used as an indicator of
216 model accuracy or quality when it should be used only to evaluate the performance of different
217 models based on the same data (Lobo et al. 2008; Yackulic et al. 2013; Fourcade et al. 2014).
218 Using a buffer to limit the locations that the model can use to randomly choose background
219 points, as we did here (see information on background points in Table A1), results in a lower
220 AUC value (VanDerWal et al. 2009, Yackulic et al. 2013). Overall response curves to individual
221 environmental predictors reflected known *Atelopus* habitat preferences (Fig. A3). The mean
222 omission rate of our model was very close to predicted omission (Phillips 2017), showing good
223 discrimination between suitable and unsuitable sites (Fig. A2). The variables that contributed

224 most to the model were annual mean air temperature (permutation importance = 66.3%) and
225 mean diurnal temperature range (11.7%) (Table A2). The map produced by the model largely
226 aligns with expectations based on our experience surveying for *Atelopus* in Panama (Fig. 2B).
227 However, two areas of predicted suitable habitat that have been well-surveyed but found not to
228 be occupied by *Atelopus* include Cerro Hoya on the Azuero peninsula and Serranía de Majé
229 which are disjunct from the central cordillera with unsuitable connecting habitat that may have
230 been a biogeographic barrier to colonization (Fig. 2B).

231

232 Taken together, the habitat suitability and distribution maps in Figure 2 will help us identify
233 areas of predicted suitability that have been poorly sampled due to inaccessibility. This
234 information will allow us to prioritize potential survey and monitoring sites.

235

236 The mapping exercise also revealed some taxonomic issues requiring attention. Firstly, the as yet
237 unnamed *Atelopus* aff. *limosus* should be investigated further and either described as a distinct
238 species or recognized as a range extension for *A. limosus*, which would then occupy the entire
239 Chagres Highlands-San Blas-Darien mountain range. Secondly, the uncommon sympatry
240 between *A. chiriquiensis* and *A. varius* was based on morphological differences (Savage 1972)
241 but would benefit from data showing genetic distinctness as well, especially given that other
242 morphologically distinct *Atelopus* species will readily hybridize in captivity (RI unpublished
243 data). The Maxent model suggests that *A. varius* could have contiguous distribution throughout
244 the central cordillera from San Jose in Costa Rica to El Cope in Panama (Fig 1 B), but observed
245 genetic differences between Costa Rican and Panamanian *Atelopus varius* (Richards and
246 Knowles 2007), may indicate that the unusually wide ranging *Atelopus varius* is actually
247 comprised of several species with more restricted distributions.

248

249 Our habitat suitability map could be used to identify areas where *Atelopus* may be persisting in
250 climate refugia (Sheele et al. this volume). Climate refugia exist in areas where a host species
251 can persist with the pathogen or where hosts persist outside of the potential distribution of the
252 pathogen (Woodhams et al. 2011). Studies suggest that amphibians can use climate refugia to
253 survive in areas with *Bd*. In Australia, *Litoria lorica* had disappeared from its known range, but a
254 population was later found persisting with *Bd* infection in another drier area (Puschendorf et al.
255 2011). *Craugastor taurus* was rediscovered in Costa Rica in an area that was drier and warmer
256 than the species' historical habitat, and these animals were persisting despite a high *Bd*
257 prevalence (Chaves et al. 2014). Rebollar et al. (2014) found that the disease burden of
258 amphibians was lower in the lowlands of Panama. They hypothesized that this was because *Bd*
259 did not grow or reproduce well in lowland climates, which allowed amphibian defenses to be
260 relatively more effective (Rebollar et al. 2014).

261

262 For *Atelopus*, chytridiomycosis-related declines in warmer, drier lowland areas tend to be slower
263 (McCaffery et al. 2015). In some places, *Bd*-related mortality may be offset by recruitment
264 (Lampo et al. 2017). Furthermore, *Atelopus* once thought to be extinct have been rediscovered in
265 places that are marginally suitable for the *Bd* (García-Rodríguez et al. 2012; Perez et al. 2014;
266 Voyles et al. 2018). Future work will use the habitat suitability model we have presented for
267 *Atelopus* and overlay it with a similar *Bd* model in Panama to identify potential climate refugia
268 that could be used as reintroduction sites. Captive breeding populations will be critical to testing

269 the climate refugia hypothesis as it will require the release of animals in multiple sites with
270 different microclimates.
271

272 An alternative reintroduction strategy could be the intentional reintroduction of large numbers of
273 animals to areas where *Bd* is present, allowing allow natural selection to act upon large numbers
274 of animals in the hopes that eventually there might be survivors. Given the large numbers
275 required, releases at the tadpole stage followed by intensive post-release monitoring would likely
276 be the most cost-effective approach. However, much more information on limiting factors in the
277 system would be needed, including potentially affecting the pathogen load in existing amphibian
278 communities by releasing highly susceptible species into them (DiRenzo et al. 2018). Other
279 uncertainties with reintroduction are not necessarily related to *Bd*, and optimal reintroduction
280 strategies depend on many variables and can vary with objectives (Canessa et al. 2014).
281 Amphibians are generally thought to be better adapted for reintroduction efforts than other
282 animals because of small body size, high fecundity, and hard-wired physiology and behavior
283 (Griffiths and Pavajeau 2008), though this does not necessarily apply to all species as life history
284 traits are variable (Tapley et al. 2015). It is not known how well captive *Atelopus* would
285 transition back into the wild, so trial releases of surplus-bred frogs could provide valuable
286 information, such whether individuals recover their wild-type microbiome or toxicity, what other
287 sources of mortality are, which life stage has the highest probability of survival in relation to
288 rearing costs. Trial releases can also provide information on the best release method (soft vs.
289 hard release) to maximize persistence and help us to improve post-release monitoring methods
290 that have notoriously low reencounter rates (Brannelly et al. 2016).

291
292 PARC has begun limited release trials with captive bred animals to begin researching these
293 issues. Five hundred surplus *A. varius* and *A. limosus* were released in 2017 and 2018. The first
294 trial evaluated holding frogs for 30 days in mesocosms (soft release) vs hard releases (direct
295 introduction to the wild) but did not detect major differences in post-release survivorship (B.
296 Klocke, A. Estrada and D. Medina, unpublished data). Released animals fitted with
297 radiotransmitters quickly dispersed out of the post-release monitoring area, resulting in low
298 recapture rates of non-radiotracked animals (B. Klocke, unpublished data), making the
299 deployment of mesocosms more attractive to guarantee re-encounters with frogs over the
300 medium term. While post-release monitoring is a difficult and resource-intensive exercise, it is
301 essential to understand the fate of reintroduced animals for use in adaptive management
302 frameworks (Canessa, This Volume; Converse, This Volume; Grant et al. 2017).

303 **3 Conclusions**

304 *Bd* is an ongoing threat to amphibian populations in Panama, and without a way to mitigate the
305 associated disease-related declines, reintroductions of amphibians are generally not
306 recommended as they are likely to end in failure (Muths and MacCallum 2016). However, robust
307 captive populations of *Atelopus* species in Panama offer a variety of opportunities to conduct
308 research that improve our knowledge of the species' ecology. Observation is the first step in the
309 scientific method and a hands-on approach may ultimately offer insights into reintroduction and
310 other management solutions (Grant et al. 2017). Captive collections serve two importance
311 conservation purposes – one immediate and one long-term. Immediately following the *Bd*
312 epidemic, captive collections has prevented the extinction of some highly susceptible species. In

313 the long-term, success in captive rearing has produced surplus individuals that can be used for
314 research to improve conservation strategies and provide individuals for reintroduction. These
315 living collections of animals are more than a simple insurance policy for threatened species, they
316 are an important resource for research that will ultimately lead to the reestablishment of
317 sustainable wild populations of these species.

318
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342
343
344 **Appendix**

345 Data Sources and Methods for Maxent model.

346 **A1 Table.** Details of settings for tuning runs and final Maxent model.

347 **A2 Table.** The permutation importance of each variable which shows the contribution of each
348 variable to the final model.

349 **A1 Fig.** Receiver operating characteristic (ROC) curve averaged over 20 runs.

350 **A2 Fig.** Test omission rate and predicted area as a function of the cumulative threshold, averaged
351 over 20 runs.

352 **A3 Fig.** Response curves showing how each environmental variable affects the model prediction.

353

Box 1. Conservation status of *Atelopus* in Panama

Also see Fig. 2A for historical ranges of all species and Table 1 for information on *Bd* declines. The Amphibian Ark recommends collecting at least 20 pairs of each species as founders with the goal of 10 breeding pairs and growing the population to 300-500 individuals (Schad 2008).

***Atelopus certus* - Status in the wild: Critically Endangered (IUCN, In press).** This species is endemic to the Darien region of Panama. *Bd* has not yet been detected in this species' range, but future population declines are projected based on declines observed for other species in this genus (La Marca et al. 2005). The last monitoring surveys in January 2016 recorded fewer frogs than expected, however, because no individuals were recorded as *Bd* positive at the time, it is uncertain whether this is due to infection with *Bd* or the drought-related to El Niño in 2016 (RI unpublished data). **Status in captivity: Secure.** The total living captive population is represented by 22 founders out of 28 individuals that were bred, and the captive population is about 350 adult animals.

***Atelopus chiriquiensis* - Status in the wild and captivity: Extinct (IUCN, In press).** This species was once considered locally abundant along streams near the border of Panama and Costa Rica but declined due to chytridiomycosis (Berger et al. 1998; Lips 1999). There have been no known sightings of this species since 1996 (La Marca et al. 2005), and experts believe the species is Extinct (Gratwicke et al. 2016). No captive populations exist.

***Atelopus glyphus* - Status in the wild: Critically Endangered (IUCN, In press).** This species was once locally abundant in the Pirre range in Panama, but the first field observations of dead, *Bd*-positive frogs were reported in 2015 (M. Ponce pers. comm. October 2015). These appear to have been linked to marked population declines as the last survey in January 2018 recorded only a single *Bd* -positive individual over three days of searching (O. A. Garcés pers. comm. May 2018). **Status in captivity: Almost secure.** Of the 20 founders bred, only 18 have surviving captive offspring, requiring at least two more unrepresented founders to be bred to meet our minimum Amphibian Ark population goals. The total adult captive population is about 350 animals.

***Atelopus limosus* -Status in the wild: Critically Endangered (IUCN, In press).** This central Panamanian species has disappeared from many known localities since *Bd* was first detected in Chagres National Park in 2009 (RI unpublished data). Declines at higher elevation sites were very rapid while the declines at a lowland site, took place over five years and a few individuals were observed in 2015 (RI unpublished data). These sites have not been surveyed since 2016, so it is not known if remnant populations exist. In 2018, wild populations persisted at several sites within the Mamoni Valley but some individuals had heavy *Bd* infections (B. Klocke, pers. comm. May 2018). **Status in captivity: Secure.** Twenty-six individuals have been bred in captivity. Offspring from just 20 of those pairs survived to adulthood and make up the current captive population. The species shows geographic variants: some populations have a muddy brown coloration similar to the type-specimens of this species (Ibáñez et al. 1995), and others have a black and green coloration with a chevron-shaped black pattern on the dorsum. The captive founding population includes only this chevron variant.

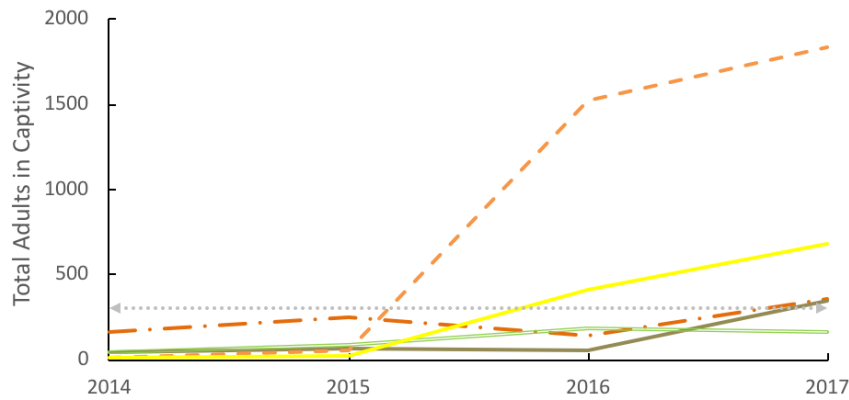
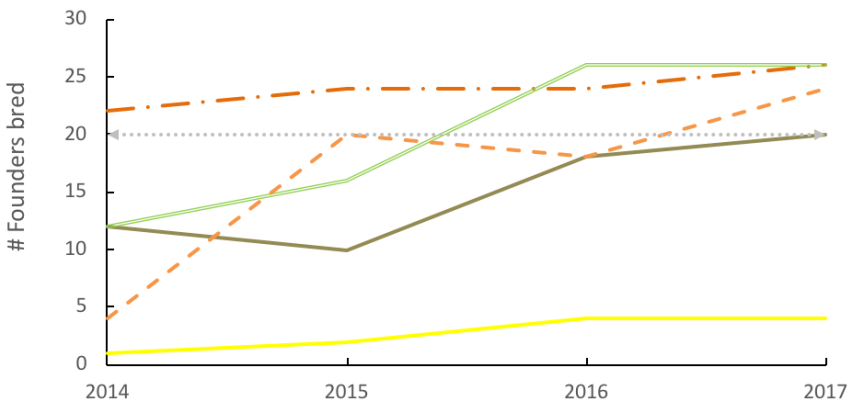
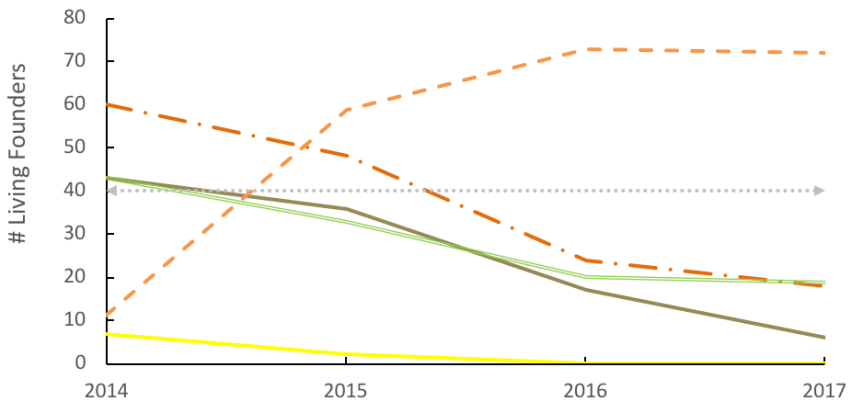
***Atelopus varius* - Status in the wild: Critically Endangered (IUCN, In press).** This species' historical range stretches along the central cordillera of Costa Rica and Western Panama (Savage 1972; Zippel et al. 2006). Most of the declines noted for this species occurred between 1987-2007. Since then, remnant populations have been rediscovered in Costa Rica (Ryan et al. 2005; Escobedo-Galván et al. 2013) and in Panama (Hertz et al. 2012; Perez et al. 2014; Voyles et al. 2018). The largest known population in Panama persists in the lowland Caribbean forests in the Donoso area but frogs there have a high *Bd* prevalence and are highly susceptible to chytridiomycosis (RI, unpublished data). **Status in captivity: Secure.** A total of 24 founders are represented in captivity in Panama including 8 highland and 16 lowland-collected founders that are separately managed. Acquisition of founders from lowland areas in the Donoso area as recently as 2016 boosted the total founder populations for this species, but many of these animals still need to be bred. In addition to the Panamanian captive population, about 160 frogs descended from six highland founders exist in U.S. zoos (K. Barrett pers. comm 2018).

***Atelopus zeteki* - Status in the wild: Critically Endangered, Possibly extinct in the wild (IUCN, In press).** This species was found around the area of El Valle de Anton and Cerro Campana (Richards and Knowles 2007). These populations have been in decline for decades due to habitat modification/loss and over-collecting for the pet trade. The first *Bd*-related declines were observed in 2005 near El Valle de Anton. The last wild animal was seen in 2009 (E. Griffith pers. comm. 2015). Surveys conducted once or twice each year between 2012 and 2017 at 4 – 6 historical sites in the El Valle area have not yet detected any persisting populations (CZ & JV unpublished data). **Status in captivity: Secure.** A total of 4 large-bodied upland founders are represented in the captive collection in Panama. The captive population in Panama is not regarded as secure from a genetic standpoint but it could be recovered through reimportation of U.S. blood-lines. More than 1,300 adult frogs descended from 32 individuals are managed by the Golden Frog Species Survival Program in the U.S. (Estrada et al. 2013; K. Barrett pers. comm. 2018). The sources of these captive populations include small-bodied lowland animals (12 founders) and larger bodied upland animals (20 founders) that are managed separately (Zippel et al 2006; Estrada et al. 2013).

***Atelopus* aff. *limosus* - Status in the wild: Not Evaluated.** This population of *Atelopus* occurs in inaccessible areas of the Darien National Park (Cerro Tarcacuna) or indigenous Comarca areas where scientific sampling permits are challenging to obtain. Further exploration is urgently needed for this population which, if it is a new species, will likely be evaluated as Critically Endangered. The frogs differ in coloration from described *Atelopus* species, but somewhat resemble the chevron color variant of *Atelopus limosus*. Genetic and taxonomic work is needed to place this population in a phylogenetic context, and disease monitoring is needed to understand if *Bd* is impacting the population. **Status in captivity: Not secure.** This species is also known from the Colombian side of the border and two males exist in captivity at the Cali Zoo, but sustainable captive populations need to be established (Flechas, et. al. 2017, S. Flechas, pers. comm. 2018).

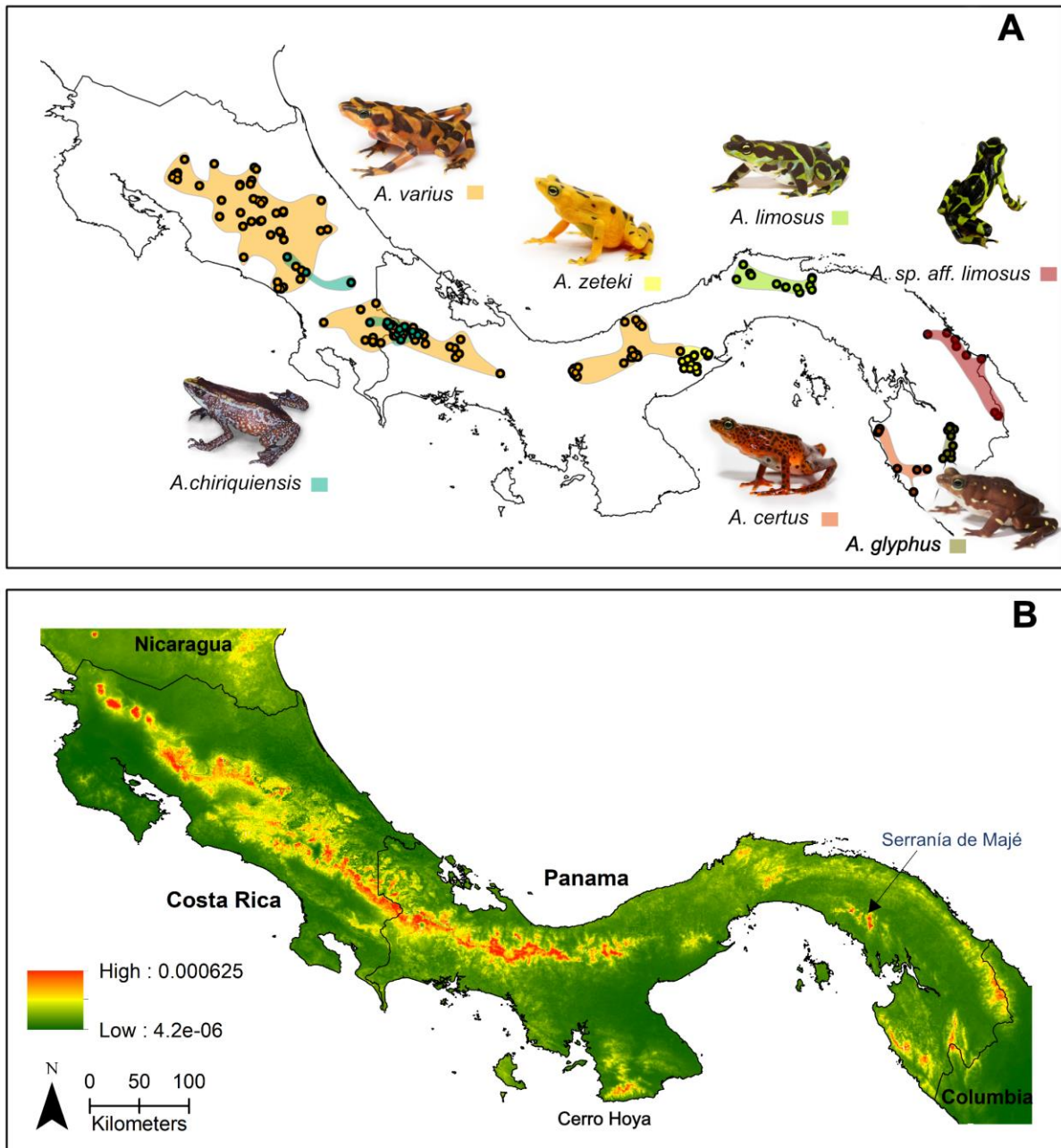
Table 1. 2018 IUCN assessment of the conservation status of wild *Atelopus* in Panama.

<i>Species</i>	IUCN Red List status (In Press)	<i>Bd</i> /declines detected	Justification
<i>A. certus</i>	CR A3ce	Not yet detected, last survey 2016 (R. Ibáñez pers comm).	> 80% future declines predicted within 21 years (the next three generation lengths). Inferred from <i>Bd</i> -related declines observed in other high altitude <i>Atelopus</i> species in the same region.
<i>A. chiriquiensis</i>	EX	1993 Las Tablas, CR (Lips et al. 2003) 1994 Cerro Pando, PA (Berger et al. 1998)	This species has not been seen since 1996, despite 2 decades of intensive searches.
<i>A. glyphus</i>	CR A4ce	2015 (M. Ponce pers. comm. October 2015)	> 80% decline inferred in 10 years since first <i>Bd</i> -related declines 2015 that are projected to continue over 21 years (three generation lengths).
<i>A. limosus</i>	CR A4ce	2009 Chagres NP, PA (R. Ibáñez pers. comm.).	> 80% decline inferred in 10 years since first <i>Bd</i> -related declines 2009 that are projected to continue over 21 years (three generation lengths).
<i>A. varius</i>	CR A4ce	1986 San Ramón, CR (Puschendorf 2003) 1987 Monte Verde, CR (Pounds and Crump 1994) 1992 Rivas, CR (Puschendorf 2003) 1997 Fortuna, PA (Berger et al. 1998) 2003 Santa Fe, PA (Brem and Lips 2008) 2004 El Cope, PA (Lips et al. 2006; McCaffery et al. 2015)	>80% decline of the known population estimated using a 21-year (3 generations) decline period window starting from 2002. Population size is unknown, but it is probably in the range of 250-2,500 mature individuals remaining in the wild.
<i>A. zeteki</i>	CR A2ace	2005 El Valle de Anton, PA (Richards-Zawacki 2010; McCaffery et al. 2015)	> 80% decline inferred in last 10 years (starting from 2008). Possibly Extinct in the wild. If any individuals remain in the wild it is unlikely that there are more than 50.
<i>A. aff. limosus</i>	NE	Not detected by Flechas et al. (2012), but no recent <i>Bd</i> surveys have been conducted (R. Ibáñez pers. comm.).	Not Evaluated, but when it becomes taxonomically recognized would likely be listed as CR A3ce using a similar justification to <i>A. certus</i> .



359 — A. certus — A. glyphus — A. limosus — A. varius — A. zeteki ◀-◌-▶ Goal

360 Fig. 1. Progress towards Amphibian Ark Captive Population Management Goals for each
 361 *Atelopus* species held in captivity in Panama between 2014-2017. The two species that are not
 362 represented in captivity are *A. chiriquiensis* and *A. aff. limosus*.
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Color should be used in print

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Fig 2. **A.** Distribution map of Panamanian *Atelopus* species. Distributions were delineated using

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occurrence records combined with visual appraisal of habitat suitability. **B.** Maxent raw habitat

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suitability map for Panamanian *Atelopus*. Each cell's suitability value is proportional to the

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expected number of presences per unit area, with all values summing to 1. The values can be

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interpreted as the relative likelihood of occurrence. Photos courtesy S.V. Flechas, M. Guerra, B.

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Gratwicke.

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625 **Online Appendix to accompany “Conserving Panamanian Harlequin Frogs by Integrating**
626 **Captive-breeding and Research Programs.”**

627

628 **Data Sources and Modeling Methods**

629 We defined our study region as 7 - 11.5 °N and 77 - 86°W, an area that encompasses all of
630 Panama and Costa Rica. We selected this region because two Panamanian species (*A.*
631 *chiriquiensis* and *A. varius*) have historic ranges extending into Costa Rica.

632

633 Species distribution models should be informed by the biotic and abiotic needs of the species.
634 Species in the *Atelopus* genus are very similar to one another in their general ecology
635 (McCaffery et al. 2015). They tend to have limited distributions and are often only known from a
636 few collection sites (Lötters 2007). They are diurnal and usually live at mid to high elevations
637 (La Marca et al. 2005). *Atelopus* are often found on stream banks or on rocks in streams in areas
638 of higher slope because they use fast-moving streams for breeding (Lötters 2007). They mostly
639 live in primary or secondary growth forests, but occasionally can be found in disturbed forest
640 bordered by cattle pasture (Lindquist and Hetherington 1998).

641

642 We gathered occurrence data for the seven species of *Atelopus* from Panama from GBIF
643 (gbif.org), VertNet (vertnet.org), the Smithsonian Tropical Research Institute
644 (stricollections.org), expedition data from the Panama Amphibian Rescue and Conservation
645 Project (RI, unpublished data), and other field efforts (CLR-Z, unpublished data). We reviewed
646 occurrence data by plotting each georeferenced locality using the ArcGIS world topo map and
647 adjusting coordinates to more accurately reflect the location description and drainage if
648 necessary. We discarded specimens with poor locality descriptions and no coordinates. The
649 distributions of most *Atelopus* species do not overlap spatially. We modified the species
650 identifications of specimens with good locality data that occurred within the known range of
651 other similar-looking species without further examination of the specimen (e.g. if a specimen
652 identified as *Atelopus varius* was documented outside the range of *A. varius* but inside the range
653 of *A. limosus*, we changed the ID to *A. limosus*). These occurrence data were used to generate a
654 species distribution map by drawing polygons around historical distribution records (Fig. 2A).

655

656 The only Panamanian *Atelopus* species that occur sympatrically are *A. chiriquiensis* and *A.*
657 *varius* (Savage 1972) in one region close to the Costa Rica-Panama border, and *A. zeteki* and *A.*
658 *varius* at a single site (Richards and Knowles 2007). In these locations, we retained the original
659 species identifications. After review, we retained 214 *Atelopus* occurrences (Fig. 2A) (*Atelopus*
660 *varius* = 111 occurrences; *A. chiriquiensis* = 27; *A. zeteki* = 21; *A. limosus* = 16; *A. glyphus* = 14;
661 *A. certus* = 11; *Atelopus* aff. *limosus* = 14). We are interested in habitat suitability for the genus
662 *Atelopus*, so the locations for all seven species were combined into one *Atelopus* occurrence
663 dataset for modeling. We made this decision because all seven species of *Atelopus* in Panama
664 occupy similar habitats. In addition, we had different numbers of occurrence records for each
665 species. The discrepancy in the number of records among species would have required different
666 modeling methods for species with few records, and this could have resulted in less robust
667 models.

668

669 We selected environmental data based on relevance to the biological requirements of *Atelopus*,
670 including climate variables, slope, and land cover. We processed all data using ArcMap 10.6 and

671 SDMToolBox 2.2 (Brown 2014) and we reprojected and resized final layers to match each
672 other and the study extent. Climate variables came from the WorldClim version 2 BioClim
673 dataset, which contains spatially interpolated monthly climate data at 1km resolution aggregated
674 from 1970-2000 (Fick and Hijmans 2017). The slope layer was created in ArcMap from the
675 Shuttle Radar Topography Mission (STRM) 1 arc-second digital elevation model (data available
676 from the U.S. Geological Survey). We obtained land cover data at 1km resolution from the
677 Global Land Cover Project North America 2000 (Latifovic et al. 2003). *Atelopus* occurrences in
678 this land cover layer matched the general description of *Atelopus* habitat from Lindquist and
679 Hetherington (1998). They were found in four land cover types, with the majority in closed
680 canopy tropical broad-leaved evergreen forest (148 occurrences). To simplify land cover into
681 biologically relevant categories for *Atelopus*, we regrouped land cover types in ArcMap to form
682 three habitat classes that correspond to *Atelopus* occurrence: classification 1 is commonly
683 occupied land cover, classification 2 combines other occupied land covers (categories 7, 18, and
684 29) and is less commonly occupied by *Atelopus*, and classification 3 combines all other
685 categories unoccupied by *Atelopus*.

686
687 We ran a pairwise Pearson's R analysis to evaluate correlation in the environmental data and
688 selected variables that were not highly correlated ($r < 7$). These variables included slope, land
689 cover, annual mean air temperature (Bio1), mean diurnal temperature range (Bio2), isothermality
690 (Bio3), annual precipitation (Bio12), precipitation of the wettest month (Bio13), precipitation of
691 the driest month (Bio14), precipitation of warmest quarter (Bio18), and precipitation of the
692 coldest quarter (Bio19). To minimize spatial bias in occurrence points, we used spatial filtering
693 to eliminate any occurrence within 1km of another, keeping as many occurrences as possible
694 (Kramer-Schadt et al. 2013; Boria et al. 2014). We chose one kilometer because *Atelopus* exhibit
695 site fidelity, have small home ranges, and are rarely found far from their stream (Crump 1986;
696 Lindquist and Hetherington 1998; Luger et al. 2009). After filtering, 166 *Atelopus* occurrence
697 records were left. *Atelopus* distribution was modeled using Maxent 3.4.1 (Phillips et al. 2006;
698 Phillips et al. 2017). To avoid overfitting, we ran tuning models based on recommendations from
699 the literature to explore settings (Merow et al. 2013) (Table S1).

700 **Table A1.** Details of settings for tuning runs and final model. For more details regarding
 701 settings, see Phillips and Dudik (2008) and Merow et al. (2013).

Setting	Description	Tuning Models	Final Model	Justification
Environmental Variables	Environmental variables used for the model. Should be relevant to the species.	Bio1, Bio2, Bio3, Bio12, Bio13, Bio14, Bio18, Bio19, Slope, Land Cover	Bio1, Bio2, Bio3, Bio12, Bio13, Bio14, Slope, Land Cover	Bio18, Bio19 permutation of importance < 1% in tuning. Variables not biologically significant in tropics as temp. stays relatively constant each season.
Feature Classes	Constraints on the model.	Linear; Linear and Quadratic; Auto	Linear and Quadratic	Linear alone did not perform well, L/Q and auto performed the same.
Regularization Parameter	Can reduce overfitting. Higher number gives more spread-out distribution.	0.5; 1.0; 2.0	1.0	Provided best habitat discrimination in output map
Background Points	Points the model compares to presence locations to differentiate more suitable environmental conditions. Should be limited to areas accessible to the species (Merow et al. 2013; Fourcade et al. 2014).	Buffered local adaptive convex-hull with alpha = 3 around occurrence points. Buffers = 10km, 15km, and 20km.	20km	Buffers biologically justified as they generally encompass environments that are similar to habitat occupied by <i>Atelopus</i> and that has been available for dispersal. 20km provided best habitat discrimination in output map
Number of Background Points	Number of points chosen by the model	10,000	10,000	More accurate results with higher numbers (Barbet-Massin et al. 2012)
Replicates	Number of times the model is run	1 time for each tuning run	20 times	Should average several runs (Barbet-Massin et al. 2012)
Training/Test partition	If one run, number set aside for training and testing the model. If multiple runs, type of replicates	75%/25%	Cross-validation with jackknife	Accounts for variation in background data

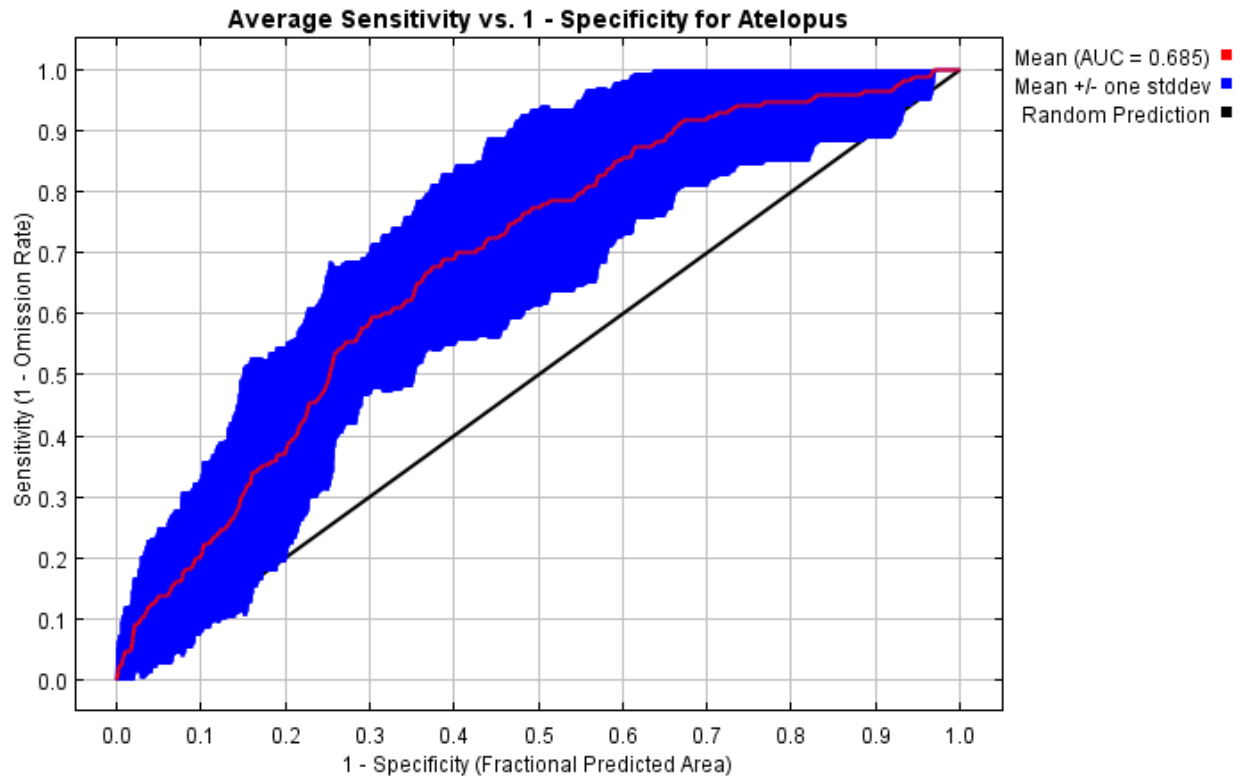
Maximum Iterations	Iterations run until model convergence	5,000	5,000	Permits convergence
Convergence Threshold	Stop training when drop in log loss equals value	0.00001	0.00001	Recommended by model
Output	Type of output from model. Commonly used are raw and logistical.	Raw	Raw	Recommended. Logistical is based on assumptions about probability of occupancy that are difficult to biologically justify (Yackulic et al. 2013).

702

703 **Table A2.** The permutation importance which shows the contribution of each variable to the
704 final model.

Variable	Permutation Importance (%)
Annual Mean Air Temperature (Bio1)	66.3
Mean Diurnal Temperature Range (Bio2)	11.7
Precipitation in Wettest Month (Bio13)	8.3
Annual Precipitation (Bio12)	6.9
Precipitation in Driest Month (Bio14)	4.5
Isothermality (Bio3)	1.1
Land Cover	0.6
Slope	0.6

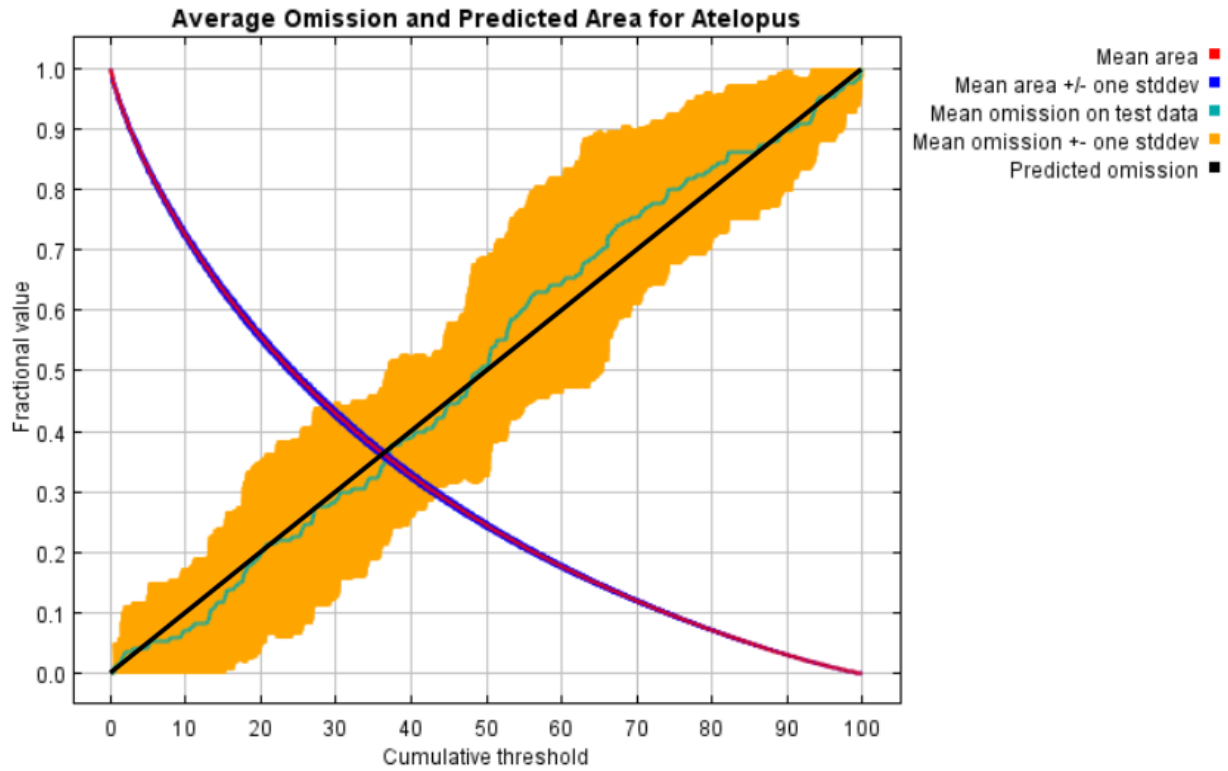
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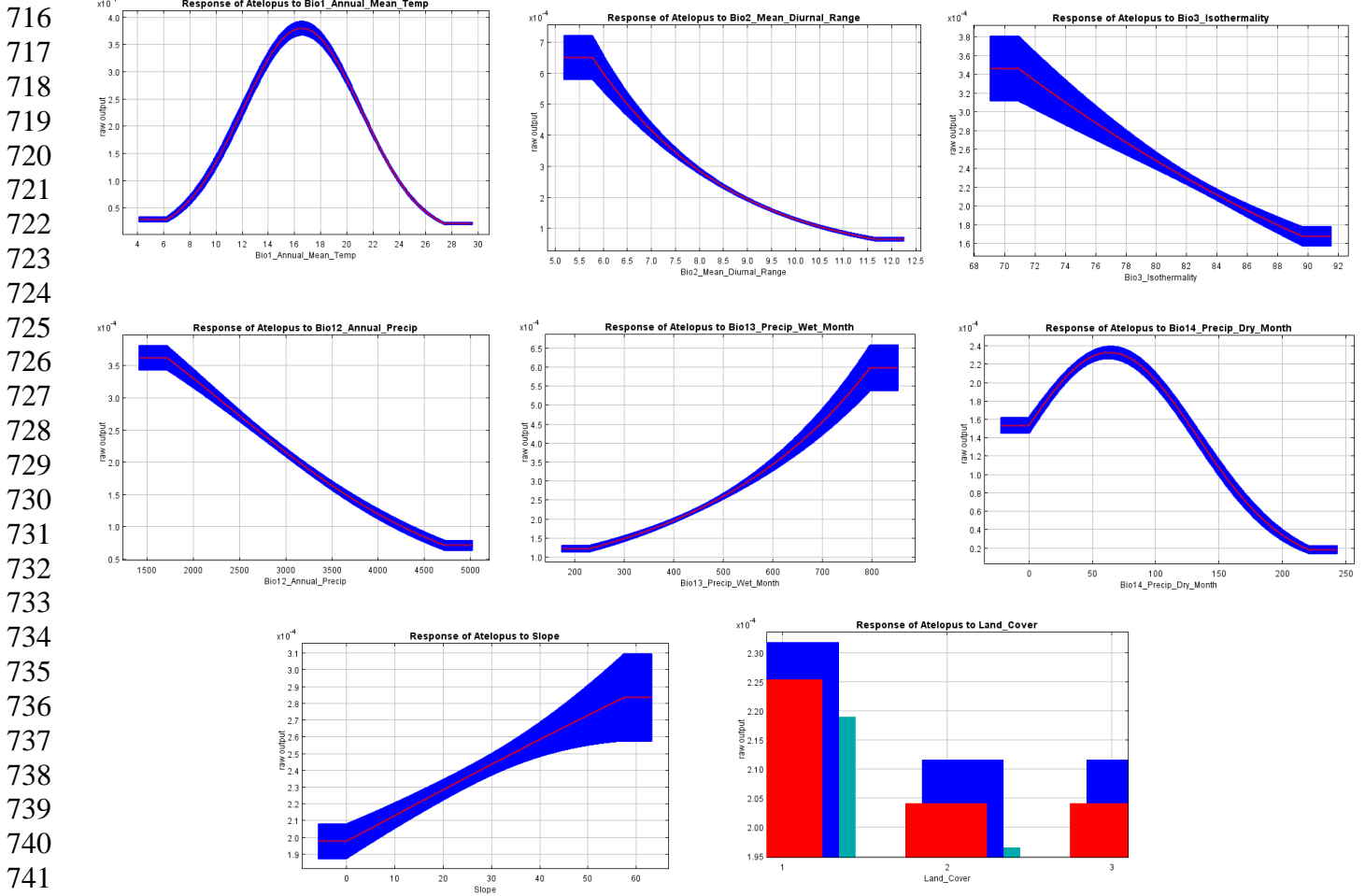
Fig. A1. Receiver operating characteristic (ROC) curve averaged over 20 runs. Average test AUC was 0.685, standard deviation 0.085.

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Fig. A2. Test omission rate and predicted area as a function of the cumulative threshold, averaged over 20 runs. Omission rate was close to predicted omission.



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 742 **Fig. A3.** Response curves showing how each environmental variable affects the model
 743 prediction. Mean response of 20 replicates in red (± 1 SD in blue). The y-axis is the raw value
 744 output estimating likelihood of occurrence. Temperature values in $^{\circ}\text{C}$, precipitation values in
 745 mm, slope value in degrees.

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750 **References for Online Appendix**

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