

Article

# Recommendations for IUCN Red List Conservation Status of the “*Dryophytes immaculatus* Group” in North East Asia

Amaël Borzée 

Laboratory of Animal Behaviour and Conservation, College of Biology and the Environment, Nanjing Forestry University, Nanjing 210037, China; amaelborzee@gmail.com

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**Abstract:** Threat assessment is important to prioritize species conservation projects and planning. The taxonomic resolution regarding the status of the “*Dryophytes immaculatus* group” and the description of a new species in the Republic of Korea resulted in a shift in ranges and population sizes. Thus, reviewing the IUCN Red List status of the three species from the group: *D. immaculatus*, *D. suweonensis* and *D. flaviventris* and recommending an update is needed. While the three species have similar ecological requirements and are distributed around the Yellow Sea, they are under contrasting anthropological pressure and threats. Here, based on the literature available, I have applied all IUCN Red List criterion and tested the fit of each species in each criteria to recommend listing under the appropriate threat level. This resulted in the recommendation of the following categories: Near Threatened for *D. immaculatus*, Endangered following the criteria C2a(i)b for *D. suweonensis* and Critically Endangered following the criteria E for *D. flaviventris*. All three species are declining, mostly because of landscape changes as a result of human activities, but the differences in range, population dynamics and already extirpated subpopulations result in different threat levels for each species. *Dryophytes flaviventris* is under the highest threat category mostly because of its limited range segregated into two subpopulations; and several known extirpated subpopulations. Immediate actions for the conservation of this species are required. *Dryophytes suweonensis* is present in both the Republic of Korea and the Democratic Republic of Korea (DPR Korea) and is under lower ecological pressure in DPR Korea. *Dryophytes immaculatus* is present in the People’s Republic of China, over a very large range despite a marked decline. I recommend joint efforts for the conservation of these species.

**Keywords:** *Dryophytes immaculatus*; *Dryophytes suweonensis*; *Dryophytes flaviventris*; Yellow Sea; North East Asia; threat; amphibian; recommendation IUCN Red List

## 1. Introduction

It is only once the threats to a species are assessed that conservation can be prioritized, and conservation planning can start [1–5]. For instance threat assessments for the Yellow-legged frog (*Rana muscosa* [6]) enabled the deployment of several threat mitigation strategies, such as trout removal [7] and translocations [8] that resulted in an increase in population size, before being impacted again by a Batrachochytrid [9]. Numerous amphibian species went extinct following the spread of the Chytrid fungus *Batrachochytrium dendrobatidis* [10] before threat assessments could be conducted as the identification of the threat and its description were not available at the time [11].

The region around the Yellow Sea basin is characterized by some of the highest human densities on earth [12–14], as well as some of the largest areas covered by rice fields [15]. This situation has very contrasting impacts on amphibians, with urbanization of landscapes having a strong negative impact

on some species [16]. Alternatively, rice paddies function as substitute habitats where species can survive (e.g., *Thamnophis gigas* [17]), or even thrive: e.g., snakes (*Oocatochus rufodorsatus* [18]), birds (*Rostratula benghalensis* [19]), bats [20], aquatic coleopterans [21] and water birds [22].

Agricultural wetlands are generally adequate surrogate habitats for amphibians as they provide regular hydroperiods that facilitate breeding, as well as regular agricultural cycles that some amphibian species can tolerate [23–29]. However, it is important to distinguish between traditional rice agriculture that can support numerous species and industrialized rice agriculture with cemented embankment and underground waterways that is not adequate for amphibian survival [30–32]. This is the case of amphibian species around the Yellow Sea where the habitat was altered by the industrialization of rice agriculture [30,31], including for instance *Dryophytes suweonensis* on the northern and eastern plains bordering the Yellow Sea and *D. flaviventris* in the Republic of Korea (hereafter, R Korea [33]), where the two species breed in rice paddies ([29,34]; Figure 1). Breeding in rice paddies is double-edged as not all agriculture phases benefit amphibians [25,35]. While the extended and regulated hydroperiod benefits tadpole development, the harvest, subsequent burning of straws and the extended fallow phase strongly impact young and hibernating individuals. In addition, transformation of natural wetlands in natural wetlands if generally very extensive, and as a result all natural wetlands within the range of the two focal *Dryophytes* species in R Korea have been transformed in agricultural wetlands, to the point that there is no population left in natural wetlands [36]. In addition, once human dietary preferences shift away from rice and rice paddies are replaced by other dry crops, then there is no habitat left for frogs, as it is suspected to be happening for *D. immaculatus* in the People’s Republic of China (hereafter, PR China [33]).



**Figure 1.** Range map for all three species from the “*Dryophytes immaculatus* group”. Ranges for the three species extracted from Borzée et al. [33]. Background map computed in ArcMap 10.6 (ESRI, Redlands, USA).

Therefore, to better understand the risks to the three species within the “*D. immaculatus* group” following the phylogenetic split and the description of *D. flaviventris* [33], I conducted threat assessments following the International Union for the Conservation of Nature Red List guidelines [37]. As of April 2020 *D. immaculatus* was listed as Least Concern [38], while *D. suweonensis* was listed as Endangered [39] and *D. flaviventris* was not yet listed. I did not include *D. japonicus* in this study despite the geographic overlap as the species is not taxonomically stable yet, with at least one additional clade in need of taxonomic clarification [40].

## 2. Materials and Methods

### 2.1. Species Introduction

The three species (Figure 2) all breed in low altitude wetlands found in alluvial plains that are near sea level, with the median altitude of locations (following the IUCN definition) equal to 3 m above sea level (ASL) for *D. suweonensis* and 1 m for *D. flaviventris* [34]. Although definitive data are not available for *D. immaculatus* yet, the species is present on slightly steeper slopes, but it is not found at medium or high altitudes (i.e., >300 m ASL [33]). *Dryophytes immaculatus* is found on the large plains surrounding the Yellow and Yangtze rivers, encompassing part of the Hebei, Shandong, Henan, Anhui and Jiangsu provinces ([41]; <http://www.amphibiachina.org>; Figure 1). The northern boundaries of *D. suweonensis* distribution are not clear yet, and while the species occurs on the plains north–east of the Yellow Sea, its distribution is restricted by either the Yalu River (bordering between PR China and the Democratic People’s Republic of Korea; hereafter, DPR Korea) or the Taeryong River between the south and north Pyongan Provinces of DPR Korea. In the south, the species ranges south until the Chilgap hills in North Chungcheong province in R Korea (Figure 1). Finally, *D. flaviventris* ranges from the Chilgap hills in the north to the Mangyeong River in the south in R Korea (Figure 1; [33]).



**Figure 2.** Pictures of the focal species in situ. (A) *Dryophytes suweonensis* (Suweon treefrog) in Asan, R Korea, June 2020; (B) *Dryophytes flaviventris* (Yellow-bellied treefrog) in Iksan, R Korea, May 2019; (C) *Dryophytes immaculatus* (Chinese immaculate treefrog) near Nanjing, PR China, June 2019. Males in (A,C) are holding onto rice seedlings while calling, the typical calling behavior of all three species. All pictures taken by author.

## 2.2. Field Surveys

The analysis I present here relies on field surveys conducted between 2013 and 2020, all following previously described protocols [34], relying on repeated aural surveys to estimate the number of calling males at each site. The results of the surveys up until 2017 are published in [42], and data on population size estimates are restricted to this period. I conducted ad hoc surveys yearly up to 2020 to determine the occurrence of the species, resulting in the addition of two isolated subpopulations (northernmost in R Korea; Figure 1), for which the total number of individuals is estimated to be approximately 40 individuals in total. To define a subpopulation as extirpated, I used historical data [43,44] and survey data, and assessed the occurrence of the species at these sites. If the number of individuals was null or I only documented a single calling individual for more than three years in a row at a site, then I considered the subpopulation to be extirpated at the site. It is not uncommon for rice paddies to be filled-in with soil by farmers to shift to dry crops, and adult individuals persist at the sites for a few years, although unable to breed. Therefore, a single calling male at a site without breeding habitat will not be able to maintain a subpopulation. In addition, it is important to highlight a potential bias in the number of individuals as the number of calling anurans at a site is generally difficult to estimate and can vary based on biotic and abiotic variables [45]. In addition, the IUCN guidelines require the total number of individuals while the surveys here are based on calling males. The bias may however be negligible as detection during repeated surveys was not significantly different between repeats within the peak breeding season [46], likely because males maintain a constant breeding range [47,48]. In addition, the population trend was confirmed by including other variables and spatiotemporal variations into analyses [49].

## 2.3. Method for Assessment

Threat assessments following the IUCN Red List categories and criteria are generally the most robust [50] and are representative of threats to a species at the global scale [51]. They are commonly used as indicators to inform on the need for conservation [52,53]. The IUCN Red List is divided into three threatened categories: Vulnerable, Endangered or Critically Endangered, and evaluations are conducted against quantitative thresholds for five criteria that determine whether a species is at risk of extinction: A, population size reduction; B, geographic range size; C, small population size and decline; D, very small population and/or restricted distribution; and/or E, quantitative analysis of extinction risk. A species that does not meet one of these criteria is placed into one of the other non-threatened IUCN categories (Least Concern and Near Threatened).

While complete datasets for the assessments are rarely available [50], alternative protocols have been established [54,55]. Assessments can then be used to assess conservation priorities (e.g., [56]), determine protected areas (e.g., [57]) and they are adequate proxies to assess biodiversity [58]. Here, I follow the IUCN Red List categories [37] and criteria to suggest the current IUCN status for each of the three focal species.

## 2.4. General Introduction and Threats to the Three Species

All threats, habitats, uses and trades are presented following the IUCN Red List criteria and categories ([www.iucnredlist.org/resources/redlistguidelines](http://www.iucnredlist.org/resources/redlistguidelines)), and in the order specified. All three species from the “*Dryophytes immaculatus* group” are impacted by residential and commercial developments in parts of their range, but they are not used or traded in any known way. The three species are found in inland wetlands and can sometimes be found in forests, especially if planted with *Salix* sp. In addition, they rely on artificial aquatic habitat such as rice paddies for breeding. *Dryophytes suweonensis* and *D. flaviventris* are not known to breed in any natural wetland anymore [36] and they are found on invasive wetland vegetation such as *Typha* sp. while resting. The three species are also known to be negatively impacted by the presence of the invasive American bullfrog (*Lithobates catesbeianus*; [46,59]) and its association with the chytrid fungus [46,60]. However, while the

chytrid fungus has been found on the focal species, its impact has not been assessed [46,61]. The two species in R Korea may also come under threat because of new invasive species, such as other *Dryophytes* species imported from North America for the pet trade [62,63].

In addition, the three species are also under similar generalized threats, such as habitat degradation and climate change. Most vertebrates have ecological preferences generally matching with those of early humans [64], which resulted in human settlements and subsequent urbanization in large alluvial plains favored by amphibians [65,66]. This is especially important for the clade studied here when considering their ecological requirements [34,67,68] and the fact that the species are found around the capitals of the three nations. While there is no evidence yet for local extirpations due to urban development for *D. immaculatus* and *D. flaviventris*, it is very likely to have happened as there are numerous large cities within the range of the two species. In the case of *D. suweonensis*, the settlement and expansion of Seoul and its metropolitan area did result in numerous documented local extirpations [34], including the type locality [43].

The three clades are impacted by climate change in similar ways. The current global warming was called to be limited to 1.5 °C above preindustrial levels by the Paris Climate Agreements [69], a challenging request unlikely to be held despite calls for more stringent regulations [70]. Even if limited to 1.5 °C above preindustrial levels, the environmental changes to the ecosystems are estimated to severely impact water resources and ecosystems, in addition to posing a moderate-to-high-risk to natural systems [71,72]. Specifically, a conservative approach taking into account the respect of the Paris Climate Agreements would result in a 2.3 °C increase in air temperature in Asia and 2.7 °C for North East Asia (peaking at 7.0 °C under a 4 °C scenario if the Paris Climate Agreements are not held; [73]). Regarding humidity, a 4.4% increase is expected in Asia and 3.3% for Northeast Asia under the 1.5 °C rise agreement (13% under a 4 °C scenario; [73]). While critical thermal maxima are not known for the focal species, experiments for other hylids [74] showed a decrease in fitness at lower temperature increases than the one predicted by the 1.5 °C rise agreement.

With regard to use of agricultural wetlands for reproduction, the increase in temperature will likely have other indirect effects. Because of higher temperatures, rice grows faster and farmers can plant rice later [75], but also flood rice paddies later, which may negatively impact the breeding ecology of the species in yet unknown ways [76]. However, because of the tight link between oviposition and flooding of rice paddies, this change in rice paddy management may delay oviposition and reduce the length of the hydroperiod available for tadpole development [29].

Finally, because of the low elevation of the habitat in relation with climate change and the rise of sea level, some subpopulations of the focal species are also under risk of being submerged [77]. A sea level rise of 60 cm would result in the direct loss of habitat for all three species (50 to 70 cm sea level rise under RCP 4.5 scenario by 2100; [78]). In addition, with sea level rise, coastal habitat will become salinized and will not be adequate for the species [79–81]. Although the species are expected to somewhat cope with low salt concentrations [33,82], predictions on the exact spread of lethal salinization inland is not currently available. However, it is likely to be widespread for *D. suweonensis* and *D. flaviventris* as 40% of the populations are on reclaimed land [34].

Based on the international database protected planet (<https://www.protectedplanet.net/>), none of the species is present in a protected area of significance. *Dryophytes immaculatus* may be found in the Yancheng UNESCO-MAB Biosphere Reserve, *D. suweonensis* is present in the Ramsar site “Mundeok Migratory Bird Reserve” in DRP Korea and *D. flaviventris* is not known to occur in any protected area. This is important to consider, as any land where the species occur can be legally developed, resulting in local extinctions.

Additional points used for further analyses: the generation times for the species are not known, but they are expected to be close to three years based on data available for *Hyla arborea* [83] and the general similarity in life history traits in Hylids [84]. An additional threat specific to *D. suweonensis* and *D. flaviventris* is the risk of hybridization [85], as well as competition with *D. japonicus* [48,86]. The breeding behavior of *D. suweonensis* and *D. flaviventris* is impacted by the calling activity of

*D. japonicus* [87] and they can be physically evicted from breeding sites in case of direct competition [87], likely because they are less bold than *D. japonicus* [88]. Moreover, *D. suweonensis* is known to be absent from sites with high level of pollution linked to agricultural practices [89].

## 2.5. IUCN Assessment: *Dryophytes immaculatus*

### 2.5.1. Population Size Reduction

The population size for the species is not known, although *D. immaculatus* may be common in suitable habitat. The species is however strongly associated with agricultural wetlands and the decrease in rice cultivation in PR China is expected to have drastically impacted the species. The area used for rice agriculture decreased by 11% since 1980 ([90,91] herein), and it can be expected to follow the same pattern over the following decades [90]) due to the decrease in water availability [92,93]. Borzée et al. [33] found “only two populations of *D. immaculatus* [ . . . ] over 49 days of field work between 2017 and 2019” at locations where the species was known to be present and abundant within the decade. Therefore, we can expect a 11% decline in population size over the last 40 years because of habitat loss and project the same decrease in population size over the next 40 years. This is however an “optimistic” estimate as the rate of development is increasing and is likely to keep on increasing, but I did not include it in this calculation.

### 2.5.2. Geographic Range

The geographic range is not accurately described and consequently the area of occupancy (AOO) and extent of occurrence (EOO) are unknown. Based on the estimate by [33], the range is divided into two disconnected areas, the one in the north being 29,000 km<sup>2</sup> and the one in the south 196,000 km<sup>2</sup> (Figure 1).

### 2.5.3. Small Population Size and Decline

The population size of the species has not been estimated, although field surveys indicated ca. 20 calling males per rice-paddy complex, a density similar to that observed for *D. suweonensis* [49]. The same work suggested a population size of 2510 calling males for a total 4671 km<sup>2</sup> (3725 + 98 + 848; range extracted from [33])—or 1.8 calling males per square kilometer.

### 2.5.4. Very Small or Restricted Population

The number of mature individuals is estimated by the thousand over a very large range, and it is unlikely to shrink by more than 11% over 40 years. Thus, this category does not apply.

### 2.5.5. Quantitative Analysis

There are no data available for a quantitative analysis, and the rate of habitat destruction over 100 years is 27.5% (11 + 11 + 5.5); as mentioned by [90] such as 11% habitat decline over 40 years.

## 2.6. IUCN Assessment: *Dryophytes suweonensis*

### 2.6.1. Population Size Reduction

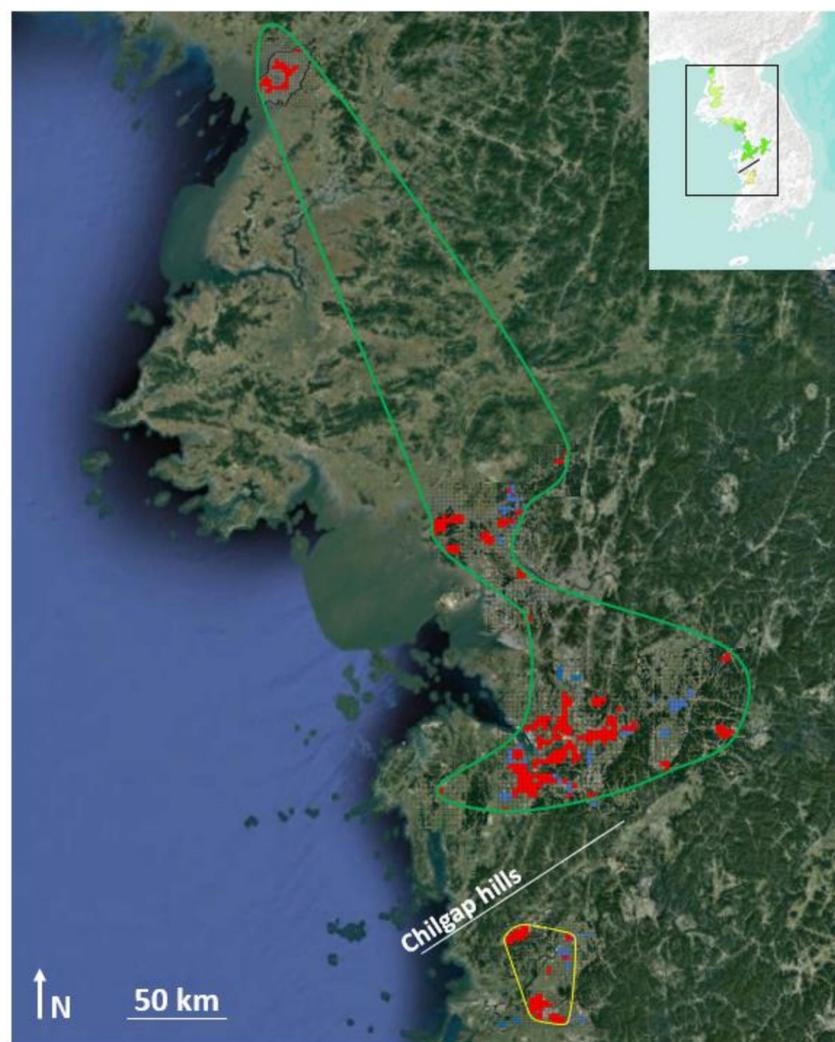
In R Korea, the population size for *D. suweonensis* + *D. flaviventris* was estimated to be an average of 2510 ± 220 calling males [49]. However, the two species were split, resulting in a total of 1986 *D. suweonensis* calling males (19 ± 24 individual per subpopulation), averaged over field surveys conducted in 2015, 2016 and 2017 (based on range from [33]). For the assessment, the rate of decline is the one determined by [49], such as −0.69 ± 1.14% of the population size over 3 years. Using this rate of decline does need to acknowledge the caveat of a likely different dynamic in DPR Korea.

However, the habitat available may be declining faster due to the rapid change in rice production. Based on data from [94] showing the land surface used for rice cultivation between 1999 and 2019, I

calculated a 1.59% decline per year (equal to an average of 16,140.05 ha). A caveat is to be mentioned as area with the highest rice productivity, and therefore less likely to be converted to another type of agriculture or be built upon, are matching with *D. suweonensis* and *D. flaviventris* population [95]. Thus, the rate of decrease in these area may be skewed, although not enough to prevent local extirpations [49].

### 2.6.2. Geographic Range

To determine the extent of occurrence (EOO) of the species, I used a minimum convex polygon on the data from [96] (Chapter 2), where the boundary of each subpopulation is highlighted. The polygon is not a perfect minimum convex polygon as I allowed one internal angle to be  $>180^\circ$  following the consideration of [97]. Some angles of the polygon are sharper than allowed by definition as they exclude the urban areas and seascapes, as per IUCN guidelines. In addition, a northernmost population in R Korea was added to the dataset, and I used the data from [33] to include the populations in DPR Korea. Here it is important to note that the IUCN definition of the EOO and area of occupancy (AOO) does not include possibly extant subpopulations. Therefore, among the subpopulations in DPR Korea only the subpopulation in Mundeok was included in this analysis (Figure 3).



**Figure 3.** Map for the area of occupancy (AOO) for *D. suweonensis* and *D. flaviventris*. Each grid cell is  $2\text{ km} \times 2\text{ km}$  as per the IUCN Red List recommendation; each cell overlapping with a subpopulation is colored in red. Cells in blue represent extirpated subpopulations. Location data extracted from [96] (Chapter 2). Map data ©2020 Google.

To calculate the AOO of the species, I transferred the polygons extracted from [96] (2018; Chapter 2) and [33] to a 2 × 2 km grid cell and determined the number of cells in which the polygons were present. For the population in DPR Korea, only the habitat matching with the ecological requirements of the species [34,67] were included in the AOO.

### 2.6.3. Small Population Size and Decline

The population size in DPR Korea was not estimated, and surveys detected a slightly higher number of calling males at the four locations surveyed in 2019 compared to that of the species in R Korea (about 40 individuals per location). Following the calculation used for *D. immaculatus*, I estimated c.1.8 calling male per square kilometer of adequate habitat. As there is 339 km<sup>2</sup> where the species is known to be present in DPR Korea, this is an additional 610 calling males. The population size in R Korea was 1986 as of 2017, with a decline of −0.69% for three years. The population size in 2020 is therefore estimated at 1986 − 13 = 1973 individuals in R Korea and 1973 + 610 = 2583 individuals on its global range.

### 2.6.4. Very Small or Restricted Population

The number of mature individuals is above 2000 individuals, this category does not apply.

### 2.6.5. Quantitative Analysis

A quantitative analysis—here a population viability analysis conducted by [96] (Chapter 13) through the use of the software Vortex v.10 (Bob Lacy; Conservation, Education and Training, Chicago Zoological Society; Brookfield, USA)—concluded that: “the results, based on an estimated original population size of 2525 individuals, showed that the population will drop below 1000 individuals within nine years, below 500 within 20 years, and the species’ probability of extinction within 100 years was 1.00. It is important to note that this analysis is presented for the total number of individuals, and not the number of calling males [96] (Chapter 13). The median time of first extinction ( $n = 1000$ ) for *D. suweonensis* was 9.98 years”. While the divergence between *D. suweonensis* and *D. flaviventris* was not known at the time of publication, data are presented per population and summarized here.

## 2.7. IUCN Assessment: *Dryophytes flaviventris*

### 2.7.1. Population Size Reduction

The number of calling males for *D. suweonensis* + *D. flaviventris* was estimated to be an average of 2510 ± 221 individuals [49]. However, the two species were split, resulting in a total of 556 calling males for *D. flaviventris* (28 ± 31 individual per subpopulation), averaged over field surveys conducted in 2015, 2016 and 2017 (based on range from [33]). Following the same calculations as for *D. suweonensis*, the decline in habitat available was estimated at 1.59% per year (equal to an average of 16,140.05 ha).

### 2.7.2. Geographic Range

I determined the EOO and calculated the AOO of the species the same way as for *D. suweonensis*. Historically, both EOO and AOO were much larger as some subpopulations are known to have been extirpated within the last decades (Figure 3 and [33]), likely due to the synergy of several pressures, which included the presence of the invasive *Lithobates catesbeianus* [46,98].

### 2.7.3. Small Population Size and Decline

The population size of the species was estimated at 556 calling males as of 2017, with a decline of 0.69% over three years. The population size in 2020 is therefore estimated at 556 − 4 = 552 calling males.

### 2.7.4. Very Small or Restricted Population

The population size was estimated at 552 calling males for 2020, a number low enough to match with some of the criteria [37].

### 2.7.5. Quantitative Analysis

The quantitative analysis conducted by [96] (Chapter 13) is used here as well, noting that numbers are presented for the total number of individuals and not the number of calling males [96] (Chapter 13). For the three independent subpopulations, the estimated time to population extinction by the PVA is such as: Buyeo  $6.82 \pm 7.08$  years, Nonsan  $4.96 \pm 6.21$  years and Iksan  $16.15 \pm 14.77$  years. As all subpopulations are now disconnected as a response to human activities, each subpopulation can be treated in isolation and the “last one alive” used as reference.

## 3. Results

### 3.1. Extinction Threat Estimate: *Dryophytes immaculatus*

#### 3.1.1. Population Size Reduction

The species is not listed as threatened based on the population size reduction variables as it has an inferred population decline below 30%, that has not stopped (criterion A1 and A2). It has an inferred population decline of 11% per 40 years, and therefore  $(11 \times 2 + 5.5)$  27.5% over 100 years, and not reaching 30% decline required to be listed under A3. As the sliding window has the same values in both past and future, the species does not qualify as threatened under A4 either (Table 1).

**Table 1.** Details of values used for the assessment. The three first row are the threshold provided in [37]. The values provided for the three *Dryophytes* species need to be read in conjunction with the corresponding time window provided in [37] and in the text (full guidelines: [www.iucnredlist.org/resources/redlistguidelines](http://www.iucnredlist.org/resources/redlistguidelines)). The data provided for each criterion is placed in the corresponding subcriteria based on variables explained in text. The population size for *Dryophytes immaculatus* is expressed with a “?” to denote the uncertainty of this value. Rows are left empty when the data for the corresponding criterion is not available.

Criterion	Critically Endangered	Endangered	Vulnerable	<i>Dryophytes immaculatus</i>	<i>Dryophytes suweonensis</i>	<i>Dryophytes flaviventris</i>
<b>A. Population size reduction</b>						
A1	≥90%	≥70%	≥50%	n. a.	n. a.	n. a.
A2	≥80%	≥50%	≥30%	27.5%	15.9%	15.9%
A3	≥80%	≥50%	≥30%	27.5%	15.9%	15.9%
A4	≥80%	≥50%	≥30%	27.5%	15.9%	15.9%
<b>B. Geographic range</b>						
B1	<100 km <sup>2</sup>	<5000 km <sup>2</sup>	<20,000 km <sup>2</sup>	>20,000 km <sup>2</sup>	18,409 km <sup>2</sup>	876 km <sup>2</sup>
B2	<10 km <sup>2</sup>	<500 km <sup>2</sup>	<2000 km <sup>2</sup>		412 + 160 km <sup>2</sup>	268 km <sup>2</sup>
<b>C. Small population size and decline</b>						
C1	<250	<2500	<10,000	405,000 ?		
C2	<250	<2500	<10,000		ca. 2500	ca. 552
<b>D. Very small or restricted population</b>						
D1	<50	<250	<1000	405,000 ?	ca. 2500	ca. 552
D2	–	–	AOO < 20 km <sup>2</sup>			
<b>E. Quantitative analysis</b>						
E	≥50%	≥20%	≥10%		50.5% R Korea	61.92%

#### 3.1.2. Geographic Range

The geographic range of the species is in excess of the 20,000 km<sup>2</sup> and the EOO and AOO are unknown for the species. Its range is not severely fragmented and not known to be fluctuating extremely, but a continued decline can be inferred for the extent and quality of habitat, likely to result

in a decrease in the number of locations or subpopulations and in the number of mature individuals. In conclusion, the species is not listed as threatened based on B1 and B2 criteria.

### 3.1.3. Small Population Size and Decline

If *D. immaculatus* is occurring at the same general population density as *D. suweonensis*, the population density would result in  $(29,000 + 196,000) \times 1.8 = 405,000$  individual *D. immaculatus*,  $\pm 10\%$  if following the same variation as the one described [49]. Here, 29,000 km<sup>2</sup> and 196,000 km<sup>2</sup> are the ranges for the southern and northern subpopulations of *D. immaculatus*. Consequently, *D. immaculatus* is not threatened under the categories C1 or C2.

### 3.1.4. Very Small or Restricted Population

The species is present in large numbers enough and over a large enough range for the categories D1 and D2 not to apply.

### 3.1.5. Quantitative Analysis

With a habitat reduction of 27.5% over 100 years as the only quantifiable data, the probability of extinction over 100 years is well above the threshold for the species to be listed as threatened under the category E.

## 3.2. Extinction Threat Estimate: *Dryophytes suweonensis*

### 3.2.1. Population Size Reduction

The population size decline is  $0.69 \pm 1.14\%$  over three years or  $2.3 \pm 3.8\%$  over 10 years, while the habitat decline is 1.59% per year, or 15.9% over 10 years. Thus, the population decline does not reach a 50% rate within 10 year to satisfy criterion A1, beside not having ceased, neither than 30% for criteria A2 to A4.

### 3.2.2. Geographic Range

For *D. suweonensis*, the EOO was estimated at 18,409 km<sup>2</sup>, including all sites with potential populations, a value below the 20,000-km<sup>2</sup> threshold of the VU category (B1). Regarding the AOO, it was estimated at 103 cells of  $2 \times 2$  km<sup>2</sup> in R Korea (412 km<sup>2</sup>; Figure 3) and 40 cells of  $2 \times 2$  km<sup>2</sup> in DPR Korea (160 km<sup>2</sup>). The species is known to have been extirpated from 54 additional cells, all in R Korea (Figure 3) There is only one recorded location in DPR Korea and there are nine independent locations in R Korea (testing criteria B2(a); [99]). Testing the criteria B2b, all locations in R Korea show a continuing observed decline of (i) extent of occurrence, (ii) area of occupancy, (iii) extent and/or quality of habitat, (iv) number of locations or subpopulations and in (v) the number of mature individuals. Regarding B2c, the number of mature individuals shows extreme variations (iv; [49]). While the species would be listed as VU at the national level: B2b(i, ii, iii, iv, v)c(iv) in R Korea and B2ab(i, ii, iii, iv, v) in DPR Korea, it is reaching the threshold of VU on its global range B2ab(i, ii, iii, iv, v)c(iv).

### 3.2.3. Small Population Size and Decline

The population size for the species is estimated at 2583 calling males on its global range, although it does not encompass location destruction, and here I round this number to 2500 calling individual. While rounding it down, it is still an optimistic number as a motorway was created along the valley linking the city of Asan and the bay of the same name, and it resulted in the destruction of rice paddies that were the habitat of the species, and also the subpopulations with some the highest count of individuals (161 calling males averaged at these locations between 2015 and 2017; [49]). In addition, the number of mature individuals in each subpopulation is well below 250 individuals and there are extreme fluctuations in the number of mature individuals [49]. Therefore, the species is listed as EN under the criterion C2a(i)b.

### 3.2.4. Very Small or Restricted Population

The species is present in large numbers enough and over a large enough range for the categories D1 and D2 not to apply.

### 3.2.5. Quantitative Analysis

The probability of extinction for *D. suweonensis* and *D. flaviventris* combined was 0.045 within 5 years, 0.134 within 10 years, 0.238 within 15 years, 0.373 within 20 years and 0.505 within 25 years. While the population in R Korea alone could qualify for EN, there is no data available for DPR Korea and a conservative estimate would consider the *D. suweonensis* population in DPR Korea to be generally similar to that of *D. flaviventris* in numbers (58 individuals in difference). Therefore, and under the biased hypothesis of similar dynamics in DPR Korea and R Korea—the species is listed as VU under the criteria E.

## 3.3. Extinction Threat Estimate: *Dryophytes flaviventris*

### 3.3.1. Population Size Reduction

The population size decline is  $0.69 \pm 1.14\%$  over three years or  $2.3 \pm 3.8\%$  over 10 years, while the habitat decline is 1.59% per year or 15.9% over 10 years. Thus, the population decline does not reach a 50% rate within 10 year to satisfy criterion A1 beside not having ceased, neither than 30% for criteria A2 to A4.

### 3.3.2. Geographic Range

For *D. flaviventris*, the EOO was estimated at 876 km<sup>2</sup>. This is below the 5000-km<sup>2</sup> threshold of the EN category B1. Regarding the AOO, it was estimated at 67 cells of  $2 \times 2 \text{ km}^2 = 268 \text{ km}^2$ , and the species is known to have been extirpated from 25 additional cells, where *Lithobates catesbeianus* is now present (Figure 3). In addition, the species is known at three independent locations only (B2(a); [99]) and all locations (B2b) show a continuing observed decline of (i) extent of occurrence, (ii) area of occupancy, (iii) extent and/or quality of habitat, (iv) number of locations or subpopulations and in (v) the number of mature individuals. Regarding the criteria B2c, the species shows extreme fluctuations in (iv) the number of mature individuals [49]. Therefore, the species reaches the threshold to be listed as EN under the criterion B2ab(i, ii, iii, iv, v)c(iv).

### 3.3.3. Small Population Size and Decline

The population size was estimated at 552 calling males for 2020. In addition, the number of mature individuals in each subpopulation is well below 250 individuals and there are extreme fluctuations in the number of mature individuals [49]. Therefore, the species is listed as EN under the criterion C2a(i)b.

### 3.3.4. Very Small or Restricted Population

The population size was estimated at 552 calling males for 2020, therefore below the 1000-individual threshold and the species is listed as VU under the criterion D1.

### 3.3.5. Quantitative Analysis

The least dramatic probability of extinction for *D. flaviventris* is  $16.15 \pm 14.77$  years, or a 50% probability of extinction within 8.075 years, equivalent to 61.92% within 10 years. The species therefore falls into the CR category under the criteria E.

#### 4. Discussion

Following the guidelines of the IUCN Red List, I argue that the three species of the “*Dryophytes immaculatus* group” in North East Asia are under similar threats, but with different severity. The main threat is habitat loss, resulting in small population sizes, to the point that *D. flaviventris* crossed the threshold to be listed as Critically Endangered (CR) under the criteria E. Next comes *D. suweonensis*, which crosses the threshold to be listed as Endangered (EN) under the criteria C2a(i)b. In addition, *D. immaculatus* was not found to meet the thresholds to be listed as threatened, however, the population size of the species is declining, and its habitat is disappearing even faster. This decline in suitable habitat is due to a decline in land used for rice agriculture, less water available, urban development within the range of the species, warmer weather, heightened salinization and the presence of predatory and competing invasive species such as the American bullfrog (*Lithobates catesbeianus*; [100]). Finally, *D. immaculatus* is very close to be listed as VU under the category A4(b,c,d,e) as the decline is a mere 2.5% below the threshold. Therefore, I recommend the species to be listed as Near Threatened (NT).

I recommend that the status of *D. immaculatus* be reevaluated in a maximum of nine years as the decrease in habitat will likely exceed the 30% threshold at that time and under the current conditions. In addition, it is very likely that this rate will increase, and no new habitat will be created for the species, thus, a shift into the VU category based on A4(b,c,d,e) within the next five years is likely. Following the IUCN Red List requirements for assessments, the conservation actions needed are the creation of protected areas and education and awareness. The situation of the species is not critical enough to recommend species management and the current laws of the PR China are sufficient to protect the species if a large enough protected area is created. In terms of research needed, the most pressing need is the exact delimitation of the species range and determining potential connectivity between populations, as the northern population could be further disjoint than currently assessed.

Regarding *D. suweonensis*, the species is currently listed as EN [39]. I recommend maintaining this listing, as had additional populations had not been found in DPR Korea, the species would have been listed as CR based on the criterion B2 following the split with *D. flaviventris*. In addition, the species is nine individuals away from reaching VU in the category A4(a,b,c) based on the results of the PVA, and it will be reaching CR within 20 years at the current rate and using the same category with a sliding window set to start 20 years from the time of publication. Accordingly, I recommend the species to be nationally listed as EN in both countries where it is found under the category B2b(i, ii, iii, iv, v), in relation with the decline in habitat and population size for R Korea. While the species should be listed as VU on its global range based on the criterion B2ab(i, ii, iii, iv, v)c(iv), a loss of 212 km<sup>2</sup> in AOO would bring it to the EN category, and additional field surveys are needed to clarify the loss of habitat since 2017, as some losses in AOO have already been recorded in R Korea. The decline in AOO for the species is alarming as the current AOO may be roughly half of the original AOO before urbanization. As the rate of population decrease is accelerating [96], the category C should be revised in 10 years, as the species will be listed as EN under the criterion C1 if the current dynamics are not corrected and CR in 40 years (based on data extracted from the PVA). I recommend the immediate protection of habitat of the species, along with other agricultural adjustments such as a limiting the height at which weeds are cut (see [101] for details). Since the populations with the highest numbers of individuals also match with the areas providing the highest rice yields [95], the conservation program should be done in concert with farmers, as neglecting rice paddies would almost certainly result in the extinction of the species. The absence of natural habitat means that without artificial flooding the species cannot breed. Species management and ex situ programs should be started as they may be the only way to save the species if nothing is done for habitat protection. In addition, incentives for treefrog friendly agriculture should be provided to farmers to help with the protection of the species.

*Dryophytes flaviventris* is characterized by a considerably narrow range that may now be half that of the pre-industrialization period. While current population decline does not warrant a threatened category, it will be reaching CR under category A4(a,b,c) within 20 years at the current rate of population

decline and using a sliding window set to start 20 years from the time of publication. The EOO of the species was estimated at 1030 km<sup>2</sup>, about ten times the threshold for CR—and contrary to the other species there is currently less pressure on the habitat—with the exception of the eastern most locations. Another characteristic of the species is the very small population size, about only three times larger than the threshold for CR and a crash in population size at the four locations with the largest population size would see the species listed as CR under the category C2a(i)b. In terms of recommendation for the species, *ex situ* programs should be started as soon as possible, and population management programs are urgently needed. A protected area including the locations with the largest population sizes must be created within a few breeding seasons and monitoring of the whole populations is urgently needed.

## 5. Conclusions

My application of IUCN guidelines for assessing the status of three species of treefrogs resulted in the recommendation of the following threat categories: Near Threatened for *Dryophytes immaculatus*, Endangered following the criteria C2a(i)b for *D. suweonensis* and Critically Endangered following the criteria E for *D. flaviventris*. The decline in these three species is strongly linked to the loss of habitat. Following changes in human diet preferences, agriculture is switching from rice to dry agriculture, resulting in a loss of habitat for these species. However, the three species are generally restricted to agricultural wetlands following the conversion of natural wetlands, and therefore the protection of the species needs to be coordinated with agricultural groups. If nothing is done, *D. suweonensis* is likely to be extirpated from most sites in the Republic of Korea within a narrow time frame and *D. flaviventris* will follow the same pattern soon afterwards.

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