Article

Preliminary Recommendations for National IUCN Red Listing Conservation Status of the "*Dryophytes immaculatus* group" in North East Asia

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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 criteria and tested the fit of each species in each criteria to recommend listing under the adequate 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 sub-populations 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 sub-populations and several known historical sub-populations are now extirpated. 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 and is under lower ecological pressure in DPR Korea. *Dryophytes immaculatus* is present in the People's Republic of China, on 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 its conservation can be prioritized, and conservation planning can start [1-5]. For instance threats assessment for the Yellow-legged frog (*Rana muscosa* [6]) enabled the deployment of several threat mitigation such as trout removal [7] and translocations [8] that resulted in a bounce back in the population size. Oppositely, numerous amphibian species went extinct following the spread of the Chytrid fungus *Batrachochytrium dendrobatidis* [9], for which threat assessments could not be conducted as the identification of the threat and its description were done in 1999 only [10].

The land around the Yellow sea basin is characterized by some of the largest human densities on earth [11-13], as well as some of the largest areas covered by rice fields [14]. This situation has very contrasting impacts on amphibians, with urbanization of landscapes having a strong negative impact on species [15]. In opposition, rice paddies can be associated with substitute habitats

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where species can survive (e.g. *Thamnophis gigas* [16]), or even thrive. Examples of species thriving in agricultural wetlands include among other snakes (*Oocatochus rufodorsatus* [17]), birds (*Rostratula benghalensis* [18]), bat guilds [19], aquatic coleopteran assemblages [20] and water birds in general [21].

Regarding amphibians, agricultural wetlands generally are adequate surrogate habitats as they provide regular hydroperiods that can be used for breeding, as well as regular agricultural cycles that some amphibian species can cope with [22-28]. It is however important to distinguish between traditional rice agriculture that can support numerous species, and industrialized rice agriculture that is not adequate for amphibian survival [29-31]. This is the case of amphibian species around the Yellow sea that saw their habitat altered by the industrialization of rice agriculture, 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 [32]), where the two species breed in rice paddies ([28, 33] Figure 1). Breeding in rice paddies is however double-edged as not all phases of agriculture benefit amphibians [24, 34] and it can result in the transformation of all natural wetlands into agricultural wetlands [35]. In additional, once dietary preferences shift away from rice and rice paddies are replaced by other dry crops, then there is no habitat left for the species as it is suspected to be happening for *D. immaculatus* in the People's Republic of China (hereafter P.R. China [32]).

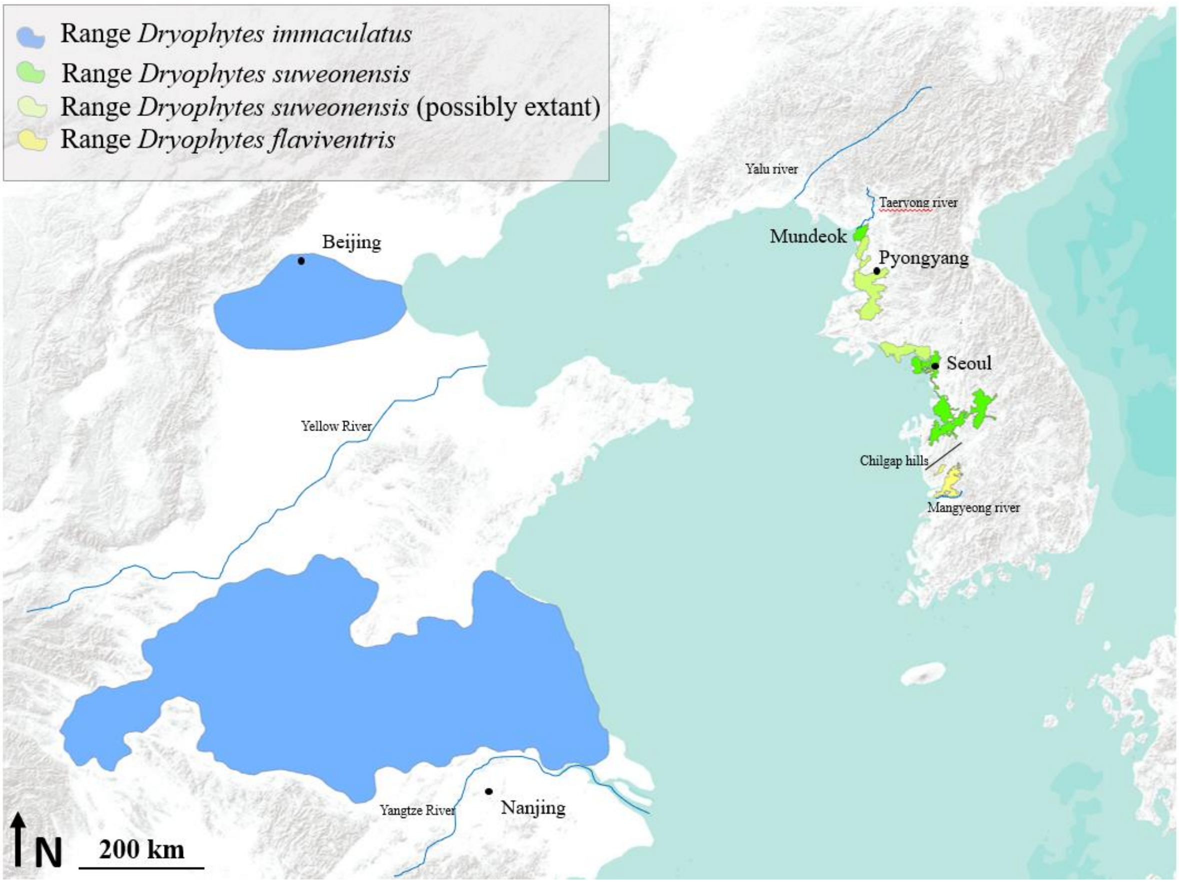

Figure 1. Range map for all three species from the "*Dryophytes immaculatus* group". The ranges for the three species is extracted from Borzée et al. (2020; [32]). Background map computed in ArcMap 10.6 (ESRI, Redlands, USA).

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* [32], I conducted threat assessments following the International Union for the Conservation of Nature Red List guidelines [36]. As of April 2020 *D. immaculatus* is listed as Least Concerned [37], while *D. suweonensis* is listed as Endangered [38] and *D. flaviventris* is not listed yet. 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 [39].

2. Materials and Methods

2.1. Species introduction

The three species breed in low altitude wetlands found in alluvial plains are strongly limited in vertical elevation, with the median altitude of locations (following the IUCN definition) equal to 3 m a.s.l. for *D. suweonensis* and 1 m for *D. flaviventris*. Although clear data is not available for *D. immaculatus*, the species is present on slightly steeper slopes but it is not found at medium altitudes [32]. *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 ([40]; 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 the R Korea (Fig.1; [32]).

2.2. Field surveys

The analysis presented here relies on field surveys conducted between 2013 and 2020, all following the protocols described in [33]. The results of the surveys up until 2017 are published in [41], 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 sub-populations (northernmost and easternmost in DPR Korea; Fig. 1), for which the total number of individuals is estimated to be upward to 40 individuals in total. To define a sub-population to be extirpated, I used historical data [42, 43] and survey data and assessed the occurrence of the species at these sites. If the number of individuals was null or a single calling individual for more than three years in a row at a site, then I considered the sub-population to be extirpated at the site. It is not uncommon for rice paddies to be filled in with soil and adult individuals are found at the sites for a few year, although unable to breed. Therefore a single calling male at a site without breeding habitat will not be able to maintain a sub-population.

2.3. Method for assessment

Threat assessments following the IUCN Red List categories and criteria are generally the most robust [44] and are representative of threats to a species at the global scale [45]. They are commonly used as indicators to inform on the need for conservation [46, 47]. 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 Concerned and Near Threatened).

While complete datasets for the assessments are rarely available [44], alternative protocols have been established [48, 49]. Assessments can then be used to assess conservation priorities (e.g. [50]; [51]), determine protected areas (e.g. [52]) and they are adequate proxies to assess biodiversity [53]. Here, I will follow the IUCN Red List categories [36] and criteria to suggest an assessment for each of the 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), and in the order specified. All three species from the "*Dryophytes immaculatus* group" are threatened by residential and commercial developments on 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 [35], 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*; [54, 55]), and its association with the Chytrid fungus [55, 56]. However, while the Chytrid fungus has been found on the focal species, its impact has not been defined [55, 57].

In addition, the three focal species are also under similar generalized threats, such as habitat degradation and climate change. Most vertebrate have ecological preferences generally matching with those of early humans [58], which resulted in human settlements and subsequent urbanization in large alluvial plains favored by amphibians [59, 60]. This is especially important for the clade studied here when considering their ecological requirements [33, 61, 62], and the fact that the species are found in the capitals of the three countries. While there is no evidence yet for *D. immaculatus* and *D. flaviventris*, the settlement and expansion of Seoul and its metropolitan area did result in numerous local extirpations [33].

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 [63], a challenging request unlikely to be held despite calls for more stringent regulations [64]. 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 [65, 66]. 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; [67]). Regarding humidity, a 4.4 % increase is expected in Asia and 3.3 % for North East Asia under the 1.5 °C rise agreement (13% under a 4 °C scenario; [67]). While critical maximal temperatures are not known for the focal species, experiments for other Hylids [68] showed a decrease in fitness through lower speed and resistance to chemical and changes in body shape under lower temperature increases than the one predicted by the 1.5 °C rise agreement.

In relation with the use of agricultural wetlands for reproduction, the increase in temperature will have other indirect effects. Because of higher temperatures, rice grows faster and farmers can plant rice later [69], but also flood rice paddies later, which will significantly impact the breeding ecology of the species in yet unknown ways [70]. However, because of the tight link between oviposition and flooding of rice paddies, it is likely to delay oviposition and reduce the length of the hydroperiod available for tadpole development [28].

Finally, because of the low elevation of the habitat in relation with climate change and the rise of sea water level, some sub-populations of the focal species are also under risk of being submerged by sea water [71]. A sea level raise by 60 cm would result in the direct loss of habitat for all three species (50 to 70 sea water rise under RCP 4.5 scenario by 2100; [72]). In addition, with sea level rise, the coastal habitat will become salinized and will not be adequate for the species [73-75]. Although the species are expected to somewhat cope with low salt concentrations [32, 76], predictions on the exact spread of lethal salinisation inland is available currently. However it is likely to be widespread for *D. suweonensis* and *D. flaviventris* as 40 % of the populations are on reclaimed land [33].

Based on the international database protected planet (https://www.protectedplanet.net/), none of the species was 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 "Rason Migratory Bird Reserve" in DRP Korea and *D. flaviventris* is not known to occur in any protected area. This is important as any land where the species are occurring can be legally developed, resulting the in the local extinction of the sub-population.

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A common point used for further analyses, the generation time for the species is not known but expected to be close to three years based on data available for *Hyla arborea* [77].

2.5. Extinction threat estimate: 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 to agricultural wetlands and the decrease is rice cultivation in PR China is expected to have drastically impacted the species. The area used for rice agriculture decreased by 11% since 1980 ([78] and [79] herein), and it can be expected to follow the same pattern over the following decades [78])due to the decrease in water availability resulting in competition [80, 81]. Borzée *et al.* [32] 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 [32], the range is divided into two disconnected area, the one in the north being 29 000 km^2 and the one in the south 196 000 km^2 (Fig. 2).

2.5.3. Small population size and decline

The population size of the species has not been estimated, although field surveys indicated c. 20 individuals per rice-paddy complex, a density similar to that observed for *D. suweonensis* [82]. The same work suggested a population size of 2 510 individuals for a total 4 671 km^2 (3 725 + 98 + 848; range extracted from [32]), or 1.8 individual per square kilometer.

2.5.4. Very small or restricted population

The number of mature individuals is estimated by the 100 of 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 is 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 [78] such as 11 % habitat decline over 40 years).

2.6. Extinction threat estimate: Dryophytes suweonensis

An additional threat to the species is the risk of hybridization [83], as well as competition with *D. japonicus* [84, 85]. *Dryophytes suweonensis* breeding behavior is impacted by the calling activity of *D. japonicus* [86] and can be evicted from breeding sites [86], likely because it is less bold than *D. japonicus* [87]. Moreover, the species is known to be absent from sites with high level of pollution linked to agricultural practices [88].

2.6.1. Population size reduction.

In R Korea, the population size for *D. suweonensis* + *D. flaviventris* was estimated to be an average of 2 510 ± 220.74 individuals [82]. However, the two species were split, resulting in a total of 1 986.67 individuals for *D. suweonensis* (19.47 ± 24.87 individual per sub-population), averaged over field surveys conducted in 2015, 2016 and 2017 (based on range from [32]). For the assessment, the rate of

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decline is the one determined by [82], 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 [89] showing the land surface used for rice cultivation between 1999 and 2019, I calculated a 1.59 % in 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 are matching with *D. suweonensis* and *D. flaviventris* population [90] and the rate of decrease in these area may be skewed, although not enough to prevent local extirpations [82].

2.6.2. Geographic range.

To determine the EOO of the species, I used a minimum convex polygon on the data from [91](chapter 2), where the boundary of each sub-population is highlighted. The polygon is not a perfect MCP as I allowed one internal angle to be > 180° following the consideration of [92]. 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 [32] to include the populations in DPR Korea. Here it is important to note that the IUCN definition of the EOO and AOO does not include possibly extant sub-populations. Therefore, among the sub-populations in DPR Korea only sub-population in Mundeok was included in this analysis (Figure 2).

To calculate the AOO of the species, I transferred the polygons extracted from [91] (2018; chapter 2) and [32] to a 2 x 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 [33, 61] 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 individuals 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 individual per square kilometer of adequate habitat. As there is 339 km^2 where the species is known to be present in DPR Korea, this is an additional 610.2 individuals. The population size in R Korea was 1 986.67 as of 2017, with a decline of -0.69 for three years. The population size in 2020 is therefore estimated at 1986.67 – 13.70 = 1972.97 individuals in R Korea, and 1972.97 + 610.2 = 2583.17 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 was conducted by [91] (chapter 13) through the use of the software Vortex v.10 (Bob Lacy; Conservation, Education, and Training, Chicago Zoological Society; Brookfield, USA), and results are such as: "the results, based on an estimated original population size of 2 525 individuals, showed that the population will drop below 1 000 individuals within nine years, below 500 within 20 years, and the species' probability of extinction within 100 years was 1.00. 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 is presented per population and summarized here.

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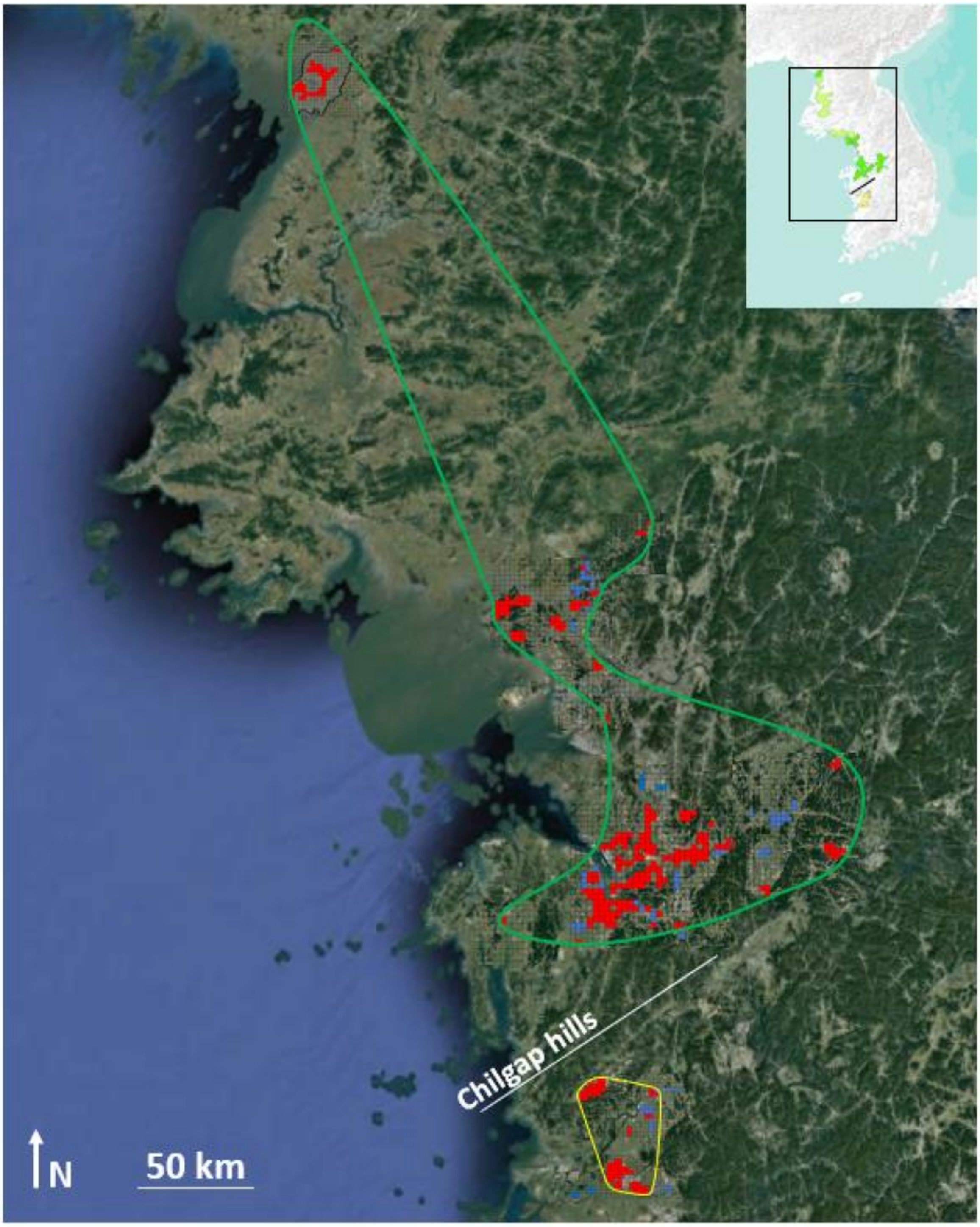

Figure 1. Map for the area of occupancy (AOO) for D. suweonensis and D. flaviventris. Each grid cell is 2 km x 2 km as per the IUCN Red List recommendation, and each cell overlapping with a sub-population has been colored in red. Cells in blue represent extirpated sub-populations. Location data extracted from [91] (chapter 2). Map data ©2020 Google.

2.7. Extinction threat estimate: Dryophytes flaviventris

2.7.1. Population size reduction.

The population size for *D. suweonensis* + *D. flaviventris* was estimated to be an average of 2 510 ± 220.74 individuals [82]. However, the two species were split, resulting in a total of 556.33 individuals for *D. flaviventris* (27.81 ± 31.06 individual per sub-population), averaged over field surveys conducted in 2015, 2016 and 2017 (based on range from [32]). Similarly to *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 would have been much larger as some sub-populations are known to have been extirpated within the last decades (Fig. 2 and [32]), likely due to the synergy of several pressures, among which the presence of the invasive *Lithobates catesbeianus* [55, 93].

2.7.3. Small population size and decline

The population size of the species was 556.33 as of 2017, with a decline of -0.69 % over three years. The population size in 2020 is therefore estimated at 556.33 – 3.83 = 552.5 individuals.

2.7.4. Very small or restricted population

The population size was estimated at 552.5 individuals for 2020, a number low enough to match with some of the criteria.

2.7.5. Quantitative Analysis

The quantitative analysis conducted by [91] (chapter 13) is used here as well. For the three independent sub-populations, the estimated time to population extinction is such as: Buyeo 6.82 ± 7.08 years, Nonsan 4.96 ± 6.21 years and Iksan 16.15 ± 14.77 years. As all sub-populations are now disconnected as a response to human activities, each sub-population 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 x 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.

3.1.2. Geographic range.

The geographic range of the species is in excess of the 20 000 km^2 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) x 1.8 = 405 000 individual *D. immaculatus*, ± 10 % if following the same variation as the one described [82]. Here, 29 000 km^2 and 196 000 km^2 are the ranges for the southern and northern sub-populations 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

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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 ± 1.14 % over three years, or 2.3 ± 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, neither than 30 % for criteria A2 to A4.

3.2.2. Geographic range.

For *D. suweonensis*, the EOO was estimated at 18 409 km^2, including all sites with potential populations, a value below the 20 000 km^2 threshold of the VU category (B1). Regarding the AOO, it was estimated at 103 cells of 2 x 2 km^2 in the R Korea (412 km^2; Fig. 2) and 40 cells of 2 x 2 km^2 in DPR Korea (160 km^2). The species is known to have been extirpated from 54 additional cells, all in R Korea (Fig. 2) There is only one recorded location in DPR Korea and there are nine independent locations in the R Korea (testing criteria B2(a); [94]). 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; [82]). 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 2 583.17 individuals on its global range, although it does not encompass location destruction, and here I round this number to 2 500 individuals. 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 sub-populations with some the highest count of individuals (161 individuals averaged at these locations between 2015 and 2017; [82]). 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 [82]. 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 conservative estivate 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 ± 1.14 % over three years, or 2.3 ± 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, neither than 30 % for criteria A2 to A4.

3.3.2. Geographic range.

For *D. flaviventris*, the EOO was estimated at 876 km². This is below the 5 000 km² threshold of the EN category B1. Regarding the AOO, it was estimated at 67 cells of 2 x 2 km² = 268 km², and the species is known to have been extirpated from 25 additional cells, where *Lithobates catesbeianus* is now present (Fig. 2). In addition, the species is known at three independent locations only (B2(a); [94]) 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 [82]. 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 552.5 individuals 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 [82]. 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 552.5 individuals for 2020, therefore below the 1 000 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 ± 14.77 years, or a 50 % probability of extinction within 8.075. The species therefore falls into the CR category under the criteria E.

4. Discussion

Following the guidelines of the IUCN Red List, I showed 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 be above any of the threshold 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 salinisation and the presence of predatory and competing invasive species such as the American bullfrog (*Lithobates catesbeianus*; [95]). 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).

Further discussion on the status of *D. immaculatus* will highlight that it will be important to re-assess the species in a maximum of nine years as the decrease in habitat will have crossed the 30 % mark 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 5 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 term of research needed, the most important point is the exact delimitation of the species range and connectivity between populations, as the northern population could be further disjoint than currently assessed.

Regarding *D. suweonensis*, the species is currently listed as EN [38]. I recommend maintain this listing as the species would have been listed as CR based on the criterion B2 following the split with *D. flaviventris* if additional populations had not been found in DPR Korea. 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^2 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 at several independent locations. The decline in AOO for the species is alarming in the country as the current AOO may be roughly half of the original AOO, before urbanization. As the rate of population decrease is accelerating [91], 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 the habitat of the species, along with other agricultural adjustments such as a limit in the height at which weeds is cut (see [96] for details). Since the populations with the highest numbers of individuals also match with the areas providing the highest rice yields [90], the conservation program should be done in concert with farmers as neglecting rice paddies would result in the near instantaneous 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 the 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 1 030 km^2, about ten times the threshold for CR, and at the contrary to the other species there is currently less pressure on the habitat, at the exception of the eastern most locations. Another characteristic of the species is the very low 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 term 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

The analyses conducted here resulted in the recommendation of the following threat categories for the three focal species: 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 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 transformation 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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