1	The range and habitat characteristics of <i>Dryophytes suweonensis</i> : the impact of land	 Deleted: Impact
2	reclamation and agricultural water regime on the conservation status of an endangered	 Deleted: distribution and
3	species	 Deleted: Dryophytes suweonensis
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13	14071, Republic of Korea.	 Comment [MOU1]: Do not see this superscript
14	⁵ Interdisciplinary Program of EcoCreative, Ewha Womans University, Seoul, 03760, Republic	
15	of Korea.	
16	* Corresponding authors	
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18	Running head: Dryophytes suweonensis range and threats	
19	Keywords: Dryophytes suweonensis, range, land reclamation, protected area, ecological	
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ABSTRACT

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Knowledge about the distribution and habitat preferences of a species is critical for its conservation. The Suweon Treefrog (Dryophytes suweonensis) is an endangered species endemic to the Republic of Korea. We conducted surveys from 2014 to 2016 at 890 potential sites across the entire range of the species. We then assessed whether landscape variables such as optimal and ancestral range, reclaimed and protected areas, and agricultural flood water affected the occurrence of D. suweonensis. Our results describe a 120 km increase in the southernmost known distribution of the species, and the absence of the species at lower latitudes. We then demonstrate a putative constriction on the species ancestral range due to urban encroachment, and provide evidences for a significant increase in its coastal range due to the colonisation of reclaimed land by the species. We also demonstrate that D. suweonensis is present in rice fields that are flooded with water originating from rivers in opposition to underground water. Finally, the non-overlap of protected areas and the occurrence of the species shows that only the edge of a single site where D. suweonensis occurs is legally protected. Based on our results and the literature, we offer a design for a site fitting all the ecological requirements of the species, and suggest the use of such site to prevent further erosion in the range of *D. suweonensis*.

Comment [REV2]: Do you mean current range? What is optimal range?

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INTRODUCTION

Very few species have a cosmopolitan distribution, and are likely to be under local environmental pressure (Purvis et al. 2000). When the entire range of a species is threatened by urbanization, or other types of habitat modification, the risk of extinction increases exponentially (Huxley 2013). As a result, the assessment of extinction risks depends on threat levels (Mace & Lande 1991; see IUCN 2016), which may guide optimal conservation effort

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to prevent extinction (Pimm et al. 2014).

Lack of knowledge of the distribution of a species has already resulted in easily-avoided extinctions. For example, the Tecopa pupfish (*Cyprinodon nevadensis calidae*) became extinct following construction of man-made structures on the Tecopa Hot Springs, the only site where the species occurred (Miller et al. 1989). Unfortunately, this information was not available at the time of construction. Knowledge of habitat preferences of species provides background information for the assessment of extinction risks (Manne & Pimm 2001), and can be used to develop spatial models of species' distribution (Corsi et al. 2000). For instance, a subspecies of Ursini's viper, *Vipera ursinii graeca*, was known to occur in Greece and at a single locality in Albania, However, eight new localities in Albania were found through landscape and climate modelling, doubling the known range of the species (Mizsei et al. 2016).

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Although critical, obtaining information about species range and habitat preferences is only a first step for any conservation effort. At risk species with clearly defined ranges still go extinct in large number, and a way to stem this loss is through the implementation of protected areas (Pimm et al. 2014). The occurrence of a species within a protected area will significantly increase its chance of survival, despite debated effects (Abellán & Sánchez-Fernández 2015), and despite the low occurrence of the endangered species within protected areas (Brooks et al. 2004).

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The amphibia class is currently the most endangered class of animals (Stuart et al. 2004).

Among the difficulties for amphibian conservation efforts are unknown distribution limits and the absence of adequate breeding sites. Suitable natural wetlands for amphibians have been converted into farmlands such as rice-paddies over the last century especially in the Reupblic of Korea (Juliano 1993; Czech & Parsons 2002; Machado & Maltchik 2010). In addition, those farmlands still holding a fraction of the original biodiversity are being converted into residential

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and commercial facilities at an alarming rate. <u>In the Republic of Korea, rice production</u> decreased by about 25 % since peak production in the 1970s (FAO 2016). Since then, there have been clear negative repercussions on habitats available for amphibians (Park et al. 2014).

The Suweon Treefrog, *Dryophytes suweonensis* (previously attributed to *Hyla*; Duellman et al. 2016), is an endangered, endemic treefrog species from the Korean Peninsula. As of 2012, the species was known to occur on a very restricted range, limited to five valleys, centred in metropolitan Seoul (Kim et al. 2012). It is therefore possible that the largest populations of *D. suweonensis* might have been historically present in and around the present Seoul area (Borzée et al. 2015a). Yet, opportunistic observations of calling males in the Democratic People's Republic of Korea (Chun et al. 2012), and further south of Seoul than previously reported (Borzée et al. 2016c), lead to expect a broader distribution for the species.

Dryophytes suweonensis is an evolutionary important species due to its unusual ZW karyotype warranting special conservation efforts (Dufresnes et al. 2015). Here, we describe the extent of occurrence and distribution of the species. Because its distribution is closely intertwined with rice cultivation, we examined whether landscape management practices such as agricultural flooding regimes, land reclamation, and the establishment of protected areas were critical for the occurrence of this species.

113 MATERIAL AND METHODS

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Field surveys were conducted during 2014, 2015 and 2016 only after the beginning of the breeding season of the species (Roh et al. 2014) to prevent any false negatives, Dryophytes suweonensis relies on rice seedlings as support from which to hang to produce advertisement calls (Borzée et al. 2016b) and typically starts breeding after rice planting.

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The setting of modern rice fields leads to a specific geometric grouping of rice paddies, here referred to as rice-paddy complexes. A rice-paddy complex is characterized by a central ditch running mostly straight through the complex for irrigation purposes. Along this central ditch, and thus along the longest and straightest line available, usually runs a cemented lane, typically following the centre of the valley. In this study, rice-paddy complexes were considered spatially independent if further than 200 m apart, the maximum daily dispersion distance for the species (Borzée et al. 2016a), or separated by landscape barriers impermeable to treefrogs (Roh et al. 2014).

The advertisement calls of *D. suweonensis* are species specific (Jang et al. 2011; Park et al. 2013), and we noted the presence or absence for *D. suweonensis* through acoustic monitoring. In calling anurans, including Hylids, acoustic monitoring is known to be reliable to estimate population size, and thus adequate to assess occurrence (Weir et al. 2005; Pellet et al. 2007; Dorcas et al. 2009; Petitot et al. 2014; Moreira et al. 2016). In a preliminary study, our aural survey protocol with 5-min transects was accurate to estimate the occurrence of *D. suweonensis* (Borzée et al. in review-a).

Transect surveys

We defined the general area for this study a priori, including all natural and man-made wetlands west of 127.5° E and below 120 m a.s.l (Roh et al. 2014). This pre-selection of potential breeding sites through Google Earth Pro (Google Earth imagery, v7.1.2.2041, 2013) identified 789 sites in 2014 (Fig. 1). A previous study for the occurrence of species had drawn the southern limit of the range around the Bay of Asan, below 37° N (Kim et al. 2012; Fig. 1). However, our surveys in 2014 demonstrated the southern limit of the range to be inaccurate (Borzée et al. 2016c), and additional surveys were conducted further south in 2015 and 2016. In 2015, we surveyed 189 sites, composed of 90 new sites and 99 sites where the species was

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present in 2014. A single site where the species was detected in 2014 could not be visited again due to its location within the Civilian Control Zone (CCZ) before the border with the Democratic Republic of Korea and the lack of permits for 2015 and 2016. In 2016, we surveyed a total of 122 sites (99 sites from 2014, 12 from 2015 and 11 new sites). All accessible sites where the species had been recorded in 2014 were surveyed in 2015 and 2016, even if the species was not detected in 2015. All sites where the species had been detected in 2015 were kept in the list of sites to survey in 2016. In total, 890 sites were surveyed at least once over the three years of surveys.

After arrival at a survey site, five minutes were spent waiting quietly. For each site, aural monitoring was conducted along a single transect from the centre of the rice-paddy complex. A surveyor walked briskly at a maximum speed of *circa* 80 m/min along the transect, noting the presence or absence of *D. suweonensis* at the rice-paddy complex. We empirically measured the detection range for advertisement calls of *D. suweonensis* (n = 20), resulting in a 250 ± 45 m range. The farthest rice paddies in rice-paddy complexes were typically within this detection range.

At the end of each transect survey we recorded water pH and water conductivity (μ S) to define the ecological preferences of *D. suweonensis*. We also estimated surface area and longest straight line within sites to determine a sphericity ratio for the occurrence of the species. We then recorded the length of continuity with rivers and forests, defined as the continuous line between the edge of rice-paddy complexes and the aforementioned landscape feature, and finally, we noted the presence of buildings and greenhouses within the rice-paddy complexes. These variables were collected through the drawing of polygons or visual inspection of sites in Google Earth Pro (Google Earth imagery, v7.1.2.2041, 2016), at a 10 m resolution, on map dated from 2015 at the latest.

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Reclaimed lands and protected areas

To correlate the presence of the species with shifting landscape use, we <u>recorded</u> the presence of *D. suweonensis* at sites located on reclaimed lands that were mudflats and sea beds that were converted into rice-paddy complexes. To record the presence of reclaimed lands, we compared maps from 1950-51 drawn by the US Army (Center of Military History 1990) downloaded in Google Earth and present satellite pictures from Google Earth Pro (Google Earth imagery, 6.2.2.6613, 2016). The 1950-51 maps were selected due to their precision. A land was considered reclaimed if it was not usable for breeding by *D. suweonensis* in 1950-51, but converted into rice paddies before 2016.

We then compared the presence of protected areas and the localities where *D. suweonensis* occurred. Data on protected areas were downloaded from the Protected Planet database, set by the IUCN and UNEP-WCMC (2016). We subsequently noted the number of sites within any protected area, as well as "sites that do not meet the standard definition of a protected area but do achieve conservation in the long-term under national and international agreements" (IUCN and UNEP-WCMC 2016).

Origin of agricultural flood waters

To analyse the impact of agricultural flood water on *D. suweonensis* distribution, we asked rice farmers for the origin of the water used to flood their rice paddies. This survey was restricted to the general riverine basin surrounding the city of Iksan, south of the Geum River. To be valid for the analysis, the origin of the water for a rice paddy complex had to be confirmed by at least two different farmers (Fig. 2). Data collection was limited to sites where surveys for *D. suweonensis* were conducted. The area surveyed south of the city of Gunsan and the Mankyeong River had to be excluded from the analysis due to lack of traceability in the origin

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of agricultural flood water (Fig. 2).

Data analysis and optimal conservation sites

For subsequent analyses, we binary encoded the presence of the species, the presence of greenhouses and the presence of permanent human infrastructures within the rice-paddy complexes. The first analysis was to determine the range of the species, based on presence data points (Fig. 1). We defined the potential range of the species and potential ancestral range, based on the non-interruption of landscape variables that are within the range used by the species. Namely, a site was considered potential for the species if < 120 m of altitude and within the same water basin as a known population, excluding cities and urban area $> 1 \text{ km}^2$ (Fig. 1).

We then defined the overlap between species range and reclaimed area to estimate the land use by the species and calculated the overlap between species range and protected areas. Descriptive statistics were used to characterise the impact of these landscape variables in both cases.

We hypothesised the origin of the water being important if linked to the Geum River. We indexed the occurrence of *D. suweonensis* at the sites surveyed in relation to the binary encoding of the origin of flood water, from the Geum river. We subsequently assessed the random distribution of *D. suweonensis* in relation of the agricultural flood water.

The last analysis for this study was the development of an optimal site for the protection of the species. From presence data from the surveys, we calculated averages for water quality (pH and conductivity) as proxies for a larger set of values important for the species (Borzée et al. in review-b), the continuity with rivers and forests, and the sphericity of sites. For sites surveyed over multiple years, the abiotic variables used for the calculation of the species preferences were restricted to the latest data point. This choice was made because of recent documented local extinctions at sites, and variables such as water quality are important the

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240 ecological preferences of a species. All analyses were conducted with SPSS (v. 21.0, SPSS, 241 Inc., Chicago, IL, USA), and maps were generated with ArcMap 9.3 (Environmental Systems Resource Institute, Redlands, California, USA). 242 243 RESULTS 244 During the surveys conducted in 2014, only 358 sites out of the 789 potential, pre-Deleted: sites selected sites were potentially habitable for the species as urban development and agricultural 245 Deleted: viable 246 conversion eliminated 431 sites. That is, these 431 sites were beyond the ecological Deleted: the Deleted: remaining 247 requirements of the species as there was no standing water but only apartment complexes, dry Deleted: or 248 crops and greenhouses. Within the 358 habitable sites, we found calling Dryophytes Deleted: Deleted: mostly 249 suweonensis at 100 sites, while the species was not detected at 258 sites. In 2015, calling D. Deleted: , despite being selected as potential sites for the occurrence of the species suweonensis were detected at 106 sites total, from 94 of the 100 sites were the species was 250 Deleted: other detected in 2014, and 12 new sites. In 2016, the species was detected at 109 sites total, 94 of 251 Deleted: of the 252 the 2014 sites, 12 of the 2015 sites and three new sites. The 94 sites originating from the 2014 Deleted: of the dataset, where the species was detected in 2015 and 2016 were the same. The species was not 253 254 detected at the five remaining sites where it had been found in 2014. The 12 sites where the species was detected in 2015 were included in the surveys in 2016, and the species was again 255 256 detected at all 12 sites. For all subsequent analyses, we assess the species to be present at the Deleted: i 257 114 sites where the species was detected at least once. This includes the 113 sites surveyed 258 three years and the site behind the CCZ. These sites are distributed over circa 4300 km² (Fig. 259 1), although under aggravated threats at the five sites where the species was detected in 2014 260 only, as a new motorway was built during the study period. Comment [MOU21]: Please clarify

Range, ancestral potential range and current optimal range

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The southern boundary of *D. suweonensis*' distribution was extended 120 km southwards from the previous assessment (Kim et al. 2012). The distribution of *D. suweonensis*

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ranges from the southern banks of the Imjin River to the northern banks of the Mankyeong River, on a 220 km north-south transect. The range of the species spans 95 km longitudinally, with the westernmost known population in Hongseong area and the easternmost in Wonju (Fig.

281 1)

The potential range of the species, defined as the area where ecological preferences of the species are matched, is situated at the same latitude as the one where the species was detected, but extends 25 km further west from the westernmost site where the species was detected, towards the reclaimed Cheonsu bay. Besides, the corridor of low lands between Nonsan, Gongju and Cheongju matches with the habitat required for the species, but no surveys were conducted in that area. When compared with the potential range of the species before human development, referred here as ancestral range, the land surface area usable by the species decreased by from 729 km² (Fig. 1).

Overlap between reclaimed lands and protected area

Out of the 114 sites where *D. suweonensis* was detected, a total of 30 sites were enlarged and 15 sites were created through land reclamation. The remaining 69 sites were not impacted by land reclamation. When combining all sites impacted by land reclamation, they represent 39.47 % of the sites where *D. suweonensis* was present. When focusing on the overlap between the occurrence of *D. suweonensis* and protected areas, only a single site was selected, South of Pyeongtaek, protected under "Water Source Protection Area". In this protected site, only the riverine system at the edge of the site is protected, putatively used by *D. suweonensis* for hibernation and not for breeding.

Origin of agricultural flood waters

A total of 53.3 % of sites where D. suweonensis was present overlapped with

Comment [REV22]: How do you establish confidence that the range will not be extended further upon further study?

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Comment [MOU24]: On map??

302 agricultural floods originating from the Geum River (Fig. 2), highlighting the non-random 303 occurrence of the species (Pearson Chi Square; $\chi 2 = 18.72$; df = 1, n = 39, P < 0.001). The 304 second most common flood water for sites with occurrence of D. suweonensis originated from Deleted: was originating the Mankyeong River, with 20.0 % of sites covered. The remaining 26.7 % of sites were 305 306 flooded by water of underground origin. Comment [REV25]: Where is the Geum and Mankyeong in relation to figure 1. Why are you zeroing in on this region? 307 Suggested conservation site 308 **Comment [REV26]:** Doesn't this belon in the discussion? 309 The environmental variables for D. suweonensis (Table 1) showed an average pH of 8.32 and average conductivity of 792.19 μS. The average sphericity was 1.15, meaning that 310 Comment [MOU27]: Explain why this is of interest earlier 311 sites were more round than elongated in general. The majority of sites where D. suweonensis occurred had permanent man-made infrastructures (52.9 %) and temporal structures (i.e. 312 313 greenhouses, 68.9 %) within the rice-paddy complexes. 314 Depiction of sites adequate for the conservation of D. suweonensis (Fig. 3) was Deleted: The drawing of the supplemented by vegetation lists from Borzée and Jang (2015), and landscape information 315 316 matching the current habitat of D. suweonensis. Rice paddies are delimited by levees roughly Deleted: with 40 cm wide and 20 to 60 cm high, covered with grasses, and used by treefrogs for basking, 317 318 foraging, and sheltering (Borzée et al. 2016a). The depiction of the designed site highlights the Deleted: bird-eye view 319 need for continuity with forests and rivers to match the preferences of the species (Fig. 3a), 320 while the lateral view (Fig. 3b) describes depth and vegetation characteristics required for the 321 species.

323 DISCUSSION

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This study highlights the importance of data on the natural history of species for their

Comment [MOU28]: Don't you mean presence/absence and habitat characteristics as opposed to natural history?

conservation. The known range of *Dryophytes suweonensis* was doubled as a result of this three year, study, leading to the need for a different approach to the selection of sites for the conservation of the species. These new data show that the increase in known range is enhanced by a large number of sites included in reclamation projects from post-war agricultural governmental development plans. Simultaneously, several potential local extirpations took place, such as all the sites in the area of Suweon where the holotype for *D. suweonensis* was described (Kuramoto 1980; Park et al. 2013).

The species still matches the criteria B1ab(i,ii,iii,iv) for listing as "endangered" under the criteria of the International Union for Conservation of nature (IUCN) red list of endangered species. Namely, because of an extent of occurrence < 5000 km², a severely fragmented population, with a continuing decline observed, estimated, inferred or projected for extent of occurrence; area of occupancy; area, extent and/or quality of habitat; and the number of locations or subpopulations. Besides, the protection of *D. suweonensis* is not ensured because no population, is located within a protected area. Only the edges of a single site are overlapping with a protected area, south of Pyeongtaek. It is however inadequate for the protection of the species during the breeding season at this site, and for the species as a whole.

The description of the potential range for *D. suweonensis* shows that an area around Cheongju may be adequate for the species to strive. However, that area was not included in the initial surveys, due to the lack of knowledge on the ecological preferences of the species.

Accordingly, sites such as Baengnyeong or Seogmo Islands could not be accessed due to their limited access to non-military personnel. Another potential significant range increase would be within the Democratic People's Republic of Korea, as the species is known to occur around Pyongyang (Chun et al. 2012).

Encroachment had been partially counter-balanced by the land reclamation projects for

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rice agriculture carried at a very large scale in the Republic of Korea during the second half of the last century. The presence of *D. suweonensis* on reclaimed land shows that the species possesses the potential for dispersal despite a lower dispersal ability, than the sympatric *D. japonicus* (Borzée & Jang 2016). This shift in range is thus linked to rice cultivation and may have been an on-going process since early human agriculture *circa* 5000 years ago (Fuller et al. 2007; Fuller et al. 2008).

Furthermore, numerous *D. suweonensis* populations are isolated from each other, with urbanization resulting in multiple landscape barriers within and among metapopulations. This calls for a long-term study on population dynamics and network analysis for the species. We would expect populations to be larger at reclaimed sites, due to lower levels of encroachment and fragmentation, in relation with preference for pristine environments by *D. suweonensis*.

The water origin analysis showed that frogs occur at sites flooded by water originating from rivers. It is, however, unclear whether treefrogs prefer water originating from rivers to underground water. The areas flooded by river water may be the ones that were seasonally flooded before landscape modifications by humans, which would provide an alternative explanation to the presence of the species at these sites. The absence of *D. suweonensis* at the site flooded by underground water could result from the ancestral absence of the species rather than direct impact of water quality. This idea is potentially supported by the absence of individuals at the only site flooded by water originating from the Geum River south of the Mankyeong River. However, as the water is brought by aerial channels, it is possible that some individuals *D. suweonensis* will drift south to this area in the future and establish new colonies, or at least hybridise with the *D. japonicus* present at the site (Borzée et al. 2015b).

Conservation of a species often requires the restoration of the species' habitat (Rannap et al. 2009). The design of an optimal site for the protection of *D. suweonensis*, is unusual as it

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highlights the need for very large continuous flood plains. However, such large plains are becoming frequently uncommon because of encroachment, and management plans have to be set before the disappearance of such sites. Furthermore, the presence of bullfrogs in the southern part on the range, in relation with their known negative impact on the species (Borzée et al. in review-a) shows that preliminary work for the protection of the species has to be conducted at any site where the species would be protected/re-introduced. Finally, as the species is still present on a range similar to its ancestral range, we do not recommend ex-situ conservation projects, nor introduction to new sites that would be outside of the species

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ancestral range.

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537 TABLES

538 Table 1:

Descriptive statistics for abiotic variables of interest collected from all sites where *Dryophytes suweonensis* was present.

	N	Min	Max	Mean	Std
Water pH	114	7.20	10.20	8.32	0.32
Water conductivity (µS)	114	83.50	5720.00	792.19	740.47
Surface area (m ²)	114	0.31	26.09	4.78	4.36
Max. length (km)	114	1.10	301.00	6.30	27.89
Continuity with forests (km)	114	0.00	14.10	3.87	2.83
Continuity with rivers (km)	114	0.00	9.20	1.17	1.79
Sphericity	114	0.01	2.87	1.15	0.65

543	FIGURES
544	Figure 1:
545	Summary of the 890 sites surveyed. Dryophytes suweonensis was detected at least once at 114
546	sites, and 421 sites were too excessively urbanised for the species to occur. Here, potential
547	range is defined as the range where the species could currently occur, while the ancestral

potential range is the range where the species could have occurred before urban development.

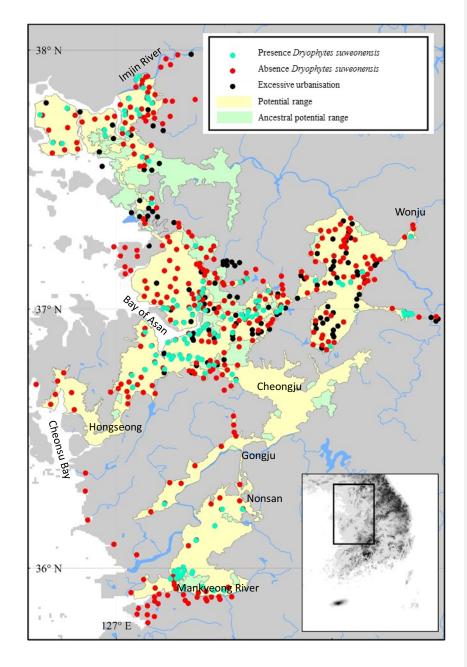


Figure 2:

Most of the flood water matching with the occurrence of *Dryophytes suweonensis* originated from the Geum River (53.3 %), followed by the Mankyeong river (20 %), while the remaining 26.7 % of sites was distributed between four types of flood water with underground origins.

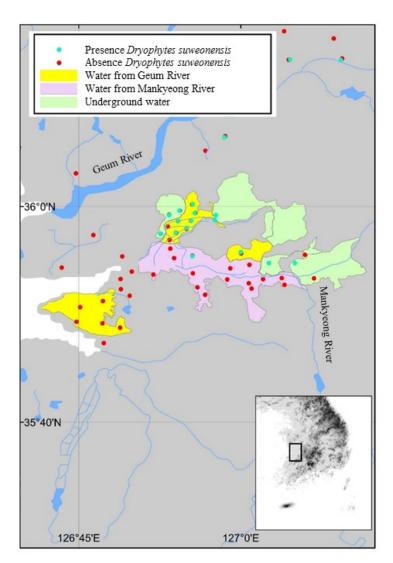


Figure 3a:

Bird view of the site optimally designed to follow ecological preferences demonstrated by

Dryophytes suweonensis. The cut AA' is reported in Fig. 3b. The figure is not to scale.

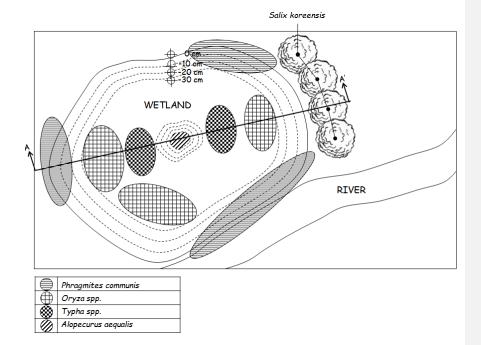


Figure 3b:

Lateral view the site optimally designed to follow ecological preferences demonstrated by *Dryophytes suweonensis*. Water depth originates from the only known natural site with *Dryophytes suweonensis* (Borzée et al., 2013).

Comment [MOU35]: Why not have both figures oriented in the same direction for visual effectiveness?

