Interspecific Variation in Seasonal Migration and Brumation Behavior in Two Closely Related Species of Treefrogs

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Most amphibians migrate between flooded habitats for breeding and dry habitats for non-breeding activities, however, differences in closely related species may highlight divergent evolutionary histories. Through field surveys, Harmonic Direction Finder tracking and laboratory behavioral experiments during the wintering season, we demonstrated differences in seasonal migration and hibernation habitats between *Dryophytes suweonensis* and *D. japonicus*. We found that *D. japonicus* migrated toward forests for overwintering and then back to rice paddies for breeding in spring. By contrast, *D. suweonensis* was found to hibernate buried in the vicinity of rice paddies, its breeding habitat. We also found that the difference in migrating behavior matched with variation in microhabitat use during brumation and hibernation between the two species. Our findings highlight different ecological requirements between the two species, which may result from their segregated evolutionary histories, with speciation potentially linked to species use of a new breeding habitat. Additionally, the use of rice paddies for both breeding and hibernation may contribute to the endangered status of *D. suweonensis* because of the degradation of hibernation sites in winter.

Keywords: brumation, *Dryophytes japonicus*, *Dryophytes suweonensis*, hibernation, hylids, migration

INTRODUCTION

Most amphibians migrate between flooded habitats for breeding and dry habitats for non-breeding activities. Differences among species in the details of these seasonal variations may highlight divergent evolutionary histories (Wake, 1982), although intraspecific variations in life-history strategies are widespread (Collins, 1981; Miaud et al., 1999), and traits covary with environmental gradients such as elevation and latitude (Morrison and Hero, 2003). In amphibians, aquatic breeding is the shared ancestral character for all species (Wake, 1982; Reiss, 2002; Schoch, 2009). Thus, migration between breeding and overwintering habitats is an evolutionary requirement tied to the biphasic life cycle of a large number of amphibians (Duellman and Trueb, 1986). The medium used for breeding typically reflects the ancestral character (Duellman, 1989), whereas migration toward different environments is a more recent evolutionary trait (Semlitsch, 2008). Exceptions do exist, however, such as for plethodontid salamanders, which retain the larval stage (Chippindale et al., 2004), and for one of the focal hylid species of this study for yet unknown reasons.

Differences in life cycles are one of the indicators of divergence in evolutionary origin between related species (West-Eberhard, 2003, 2005). Novel phenotypes stem from the reorganization of

ancestral phenotypes, followed by the genetic accommodation of changes (Mayr, 1963; West-Eberhard, 2005). Selection acts on phenotypes (Mayr, 1963), and thus populations subjected to differential environmental pressure may see the apparition of specific phenotypes that are subsequently integrated into genotypes (West-Eberhard, 2005). This includes for instance the tropical vine *Monstera* sp., which displays varying leaf forms, resulting in species-specific ontogenies (Madison, 1977). It is also the case with role-reversed sandpipers species where males incubating eggs show the same increase in prolactin as incubating females (Beach, 1961; Oring et al., 1986). Another example is the interspecific variation in parental care due to ecological requirements in *Microtus* spp. (West-Eberhard, 2003). However, selected phenotypes may arise from the pressure exerted by environmental drivers. For instance, fisheries result in the selection of individuals through both direct and indirect pressures (Heino and Godø, 2002). Direct selection pressure includes elevated mortality of target species, while indirect selection is exemplified by ecosystem-level impacts as intakes result in depleted resources (Kaiser and De Groot, 2000). Similarly, phenotypic plasticity is one of the reasons for the expression of different phenotypes between populations. For instance, local adaptation to climate can result in different range dynamics (Atkins and Travis, 2010).

Seasonal migration is only one type of population displacement (Semlitsch, 2008; Cayuela et al., 2018), but it is the most common non-circadian migration, observed from whales (Kenney et al., 2001) to butterflies (Brower, 1995). Seasonal migration is also common in amphibians (Sinsch, 1990; Ryan and Semlitsch, 1998); for instance, *Bufo bufo* hibernates in terrestrial hibernacula (Van Gelder et al., 1986; Denton and Beebee, 1993) and migrates to water bodies to breed in spring (Gittins, 1983). Some amphibian species will migrate considerable distances from their breeding site to find shelter against climatic variation (Griffiths, 1984), although 15 km is considered the limit for direct migration and dispersion due to physiological requirements (Sinsch, 1990; Cayuela et al., 2018). A representative assessment of eight Central European amphibian species demonstrated migration distances between roughly 100 and 2,200 m from the breeding site (Kovar et al., 2009). Other species, such as *Lithobates catesbeianus*, hibernate in the vicinity of their breeding sites and thus do not require migration (Stinner et al., 1994). Migration is generally observed between breeding and over-wintering sites, where species will shelter from inclement condition through torpor. While over-wintering strategies are diverse (Storey and Storey, 2017), and include under-water (Penney, 1987) and below-ground sheltering (Borzée et al., 2018c), information about brumation or hibernation for a large number of species is still missing. Here, brumation refers to the pre-hibernation period, and although yet poorly described in metabolic terms in amphibians (Feder and Burggren, 1992; Balogová et al., 2017; Wilkinson et al., 2017; Kundey et al., 2018) with the exception of *Salamandra salamandra* (Catenazzi, 2016), it refers to the pre-winter reduced activity and reduced metabolic rates of poikilotherms (Hutchinson, 1979; Pratihar and Kundu, 2011; McEachern et al., 2015). When focusing on over-wintering in treefrogs, field

observations provide the most reliable data for European species (reviewed by Stumpel, 1990), complemented by behavioral ecology studies on North American species (Mahan and Johnson, 2007; Johnson et al., 2008). In addition, some Indian species are known to shelter from the cold in banana stems (Iangrai, 2011). *Dryophytes japonicus* in North East Asia is comparatively well studied and known to start hibernating because of the rise of *cirp* RNA due to cold and photoperiod (Sugimoto and Jiang, 2008). In addition, the species is able to withstand massive temperature drops, down to −53°C in laboratory settings (Berman et al., 2016), and to principally use forested hills for hibernation (Borzée et al., 2018c).

In amphibian species in the Republic of Korea, both aquatic and dry types of non-freeze-resistant hibernation types are known. For instance, *Rana* spp. can hibernate under water (Lee and Moon, 2011; Macias et al., 2018) and are the first species present at the breeding sites after ice thaw (Yoo and Jang, 2012; Macias et al., 2018). In contrast, *Dryophytes japonicus* hibernates under decaying vegetation on hills principally forested by oak trees (Borzée et al., 2018c). It is unclear if the other treefrog species from the peninsula, the endangered *D. suweonensis*, can follow the same pattern. Unlike *D. japonicus*, the species is not found in forests during the non-breeding season. Furthermore, there are anecdotal observations of *D. suweonensis* hibernating in the banks of rice paddies (Pers. Comm. Kim Hyun-Tae). *Dryophytes suweonensis* is known to have originally bred in low-altitude alluvial wetlands, but it is now restricted to rice paddies (Borzée and Jang, 2015), whereas *D. japonicus* breeds in a much wider range of environments as long as solid substrate is available for call production (Borzée et al., 2016b). In addition, the two species display microhabitat segregation during the production of advertisement calls, likely due to competition (Borzée et al., 2016b). Given the long list of traits linked to the species' breeding behavior (Roh et al., 2014; Borzée and Jang, 2015; Borzée et al., 2015a, 2016a, 2017a; Kim, 2015b, 2016), we hypothesize that *D. suweonensis* hibernates in rice paddies, where it also breeds, whereas *D. japonicus* is expected to migrate seasonally between breeding sites and the forests hills where it hibernates. Here, we tested the hypothesis through winter field surveys of the two *Dryophytes* species. Interestingly, the pattern hypothesized is known to be similar to the one displayed in other amphibians where one of the two closely related species is restricted to rice paddies (*Pelophylax nigromaculatus* and *P. porosus brevipodus* in Japan Maeda and Matsui, 1993). The absence of seasonal migration may indicate that the two species share the same breeding and overwintering habitats, and thus exploit the same environment, whereas differences in seasonal migration would suggest a different evolutionary history and the use of different environments.

MATERIALS AND METHODS

This project is composed of 5 distinct sections consisting of experiments, field tracking and observations for both *Dryophytes suweonensis* and D. japonicus: (1) field observations for brumation, (2) field orientation tracking for brumation, (3)
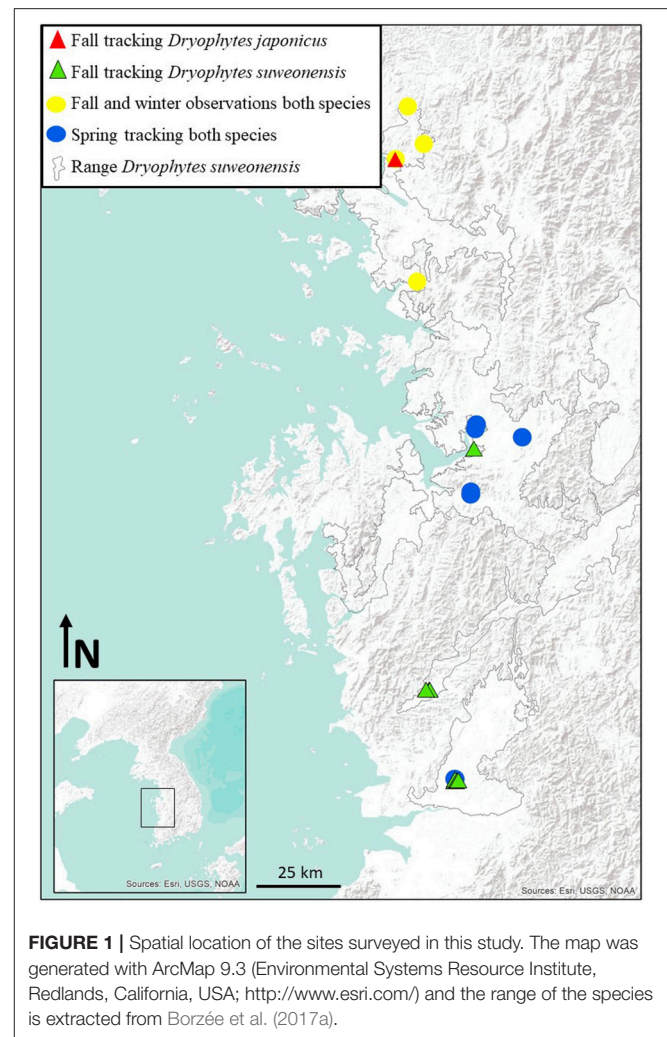
laboratory brumation and hibernation observations, (4) winter field observations and finally, (5) spring orientation tracking. Here, we define brumation as the pre-hibernation period during which amphibians are partially active (Mayhew, 1968; Pratihar and Kundu, 2011; McEachern et al., 2015), and such as defined for one of the two focal species, D. japonicus, by Borzée et al. (2018c). The five sections used to distinguish between experiments are based on the seasonal succession of activities, from pre-hibernation to emergence from hibernation. We conducted all experiments with the agreement of the Ministry of Environment from the Republic of Korea under permit numbers 2013-16, 2014-04, 2014-08, 2014-20, 2015-3, 2015-4, 2015-6, 2015-28, and 2016-5.

Dryophytes suweonensis is slender and smaller than *D. japonicus* (Borzée et al., 2013), and the earlier species is active earlier in the afternoon than the latter, although both species are principally active at night (Borzée et al., 2016b). Dryophytes japonicus is widespread on the Asian mainland until central Mongolia and the Baikal lake region in Russia (Dufresnes et al., 2016; Kuzmin et al., 2017) but the two species only co-occur on the western lowlands of the Korean Peninsula, where the distribution of D. suweonensis is restricted to agricultural wetlands due to widespread habitat modification (Roh et al., 2014; Borzée and Jang, 2015; Borzée et al., 2015a, 2018a; Borzée and Seliger, 2018). The use of rice paddies impacts the breeding behavior of both species (Borzée et al., 2018b; Groffen et al., 2018), which display both temporal and spatial segregation during the breeding season (Borzée et al., 2016a,b). The population decline of *D. suweonensis* is principally linked to habitat loss (Borzée, 2018; Borzée et al., 2018a), but other factors such as hybridization (Borzée and Jang, 2018), sensitivity to water quality (Borzée et al., 2018f), behavior (Borzée et al., 2018g), and invasive species and pathogens (Borzée et al., 2017b) are involved (Borzée et al., 2017a; Borzée, 2018).

Field Observations for Brumation

We collected field observations on the brumation ecology of the two species at two localities in 2014 ($n = 29$) and four in 2015 ($n = 32$; **Figure 1**). The two localities from 2014 (#1 and #2; **Figure 1**) are included within the four localities from 2015 (together with remaining sites #3 and #4; **Figure 1**). Locality 1 is composed of one paddy site and two forested sites, and localities 2, 3, and 4 are each composed of one paddy site and a single forested site. We selected the localities following observations of calling males of both *Dryophytes* species during the breeding season (see Borzée and Jang, 2015).

We initiated the surveys in September of both years and continued until the first freeze. In 2014, we surveyed the sites in the first and third weeks of September, every week in October and the first week of November. In 2015, we surveyed all sites in the third week of September, the first and third weeks of October and the first week in November. We conducted the surveys through spotlight line transects (Smith and Nydegger, 1985), by which the researcher followed a predetermined transect and visually inspected the vegetation for individuals. The transects at the rice paddy sites were conducted for varying distances along the straight cement road at the center of rice-paddy complexes

FIGURE 1 | Spatial location of the sites surveyed in this study. The map was generated with ArcMap 9.3 (Environmental Systems Resource Institute, Redlands, California, USA; http://www.esri.com/) and the range of the species is extracted from Borzée et al. (2017a).

(**Figure 2**; Borzée and Jang, 2017). We conducted the transects at the forest sites along a 250-m approximately straight line due to the topology of the field. Each hylid frog found was hand caught, and the species identified based on morphology (Borzée et al., 2013) when calls were not available (Park et al., 2013). We detected *D. suweonensis* during 20 surveys, and *D. japonicus* during 29 surveys.

Field Orientation Tracking for Brumation

For all microhabitat use and directionality experiments in this study, we used a Harmonic Direction Finder (HDF; R2 RECCO AB; Lidingö, Sweden), relying on a passive dipole attached to the individual to be tracked with a gauze waist band. The HDF emits microwaves toward the dipole, which bounces them back through an antenna tailored for each individual and encodes for directionality and distance (Pellet et al., 2006; Pašukonis et al., 2014; Borzée et al., 2016b). We soldered a Schottky diode (model R2 RECCO AB; Lidingö, Sweden) on a tin-plated copper wire folded on itself at 180° in a fashion that created a loop 1 cm away from the bent, prolonged by two isolated segments
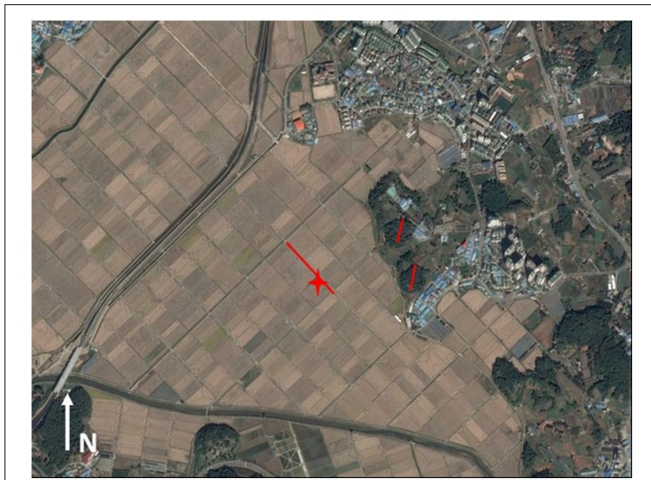
FIGURE 2 | Example of the brumation and winter field observations at site #1, Sihung, located 37.406°N and 126.805°E. The red lines represent the transects, in the rice paddies in the west and in the forests in the east. The red star indicates the site where the hibernating female was found during the winter field observations. This map was generated with Google Earth Pro (v7.1.2.2041, 2013) on maps from 2016 SKEnergy, Image Landsat Copernicus.

of the wire, resulting in an antenna. This design maintains the electric properties of the diode and provides mechanical elasticity in the antenna. The Schottky diode reflects the wave received at twice its frequency (see de Moura Presa et al., 2005; and Borzée et al., 2016b for details), which the HDF then translates into an acoustic signal of varying intensity in function of the direction and distance to the dipole, thus allowing for locating the organism that is wearing the antenna. To isolate the electrical dipole, we uniformly insulated each antenna with a silicone spray (NABAKEM, S-830 UL94 V-0, Seoul, Korea).

We selected the initial antennae with two 8-cm legs, resulting in an approximately 25-m effective range. We prepared the waistbands to which the antennae were attached with different lengths for each individual to ensure that each antenna weight was below the recommended 5% of each individual's body mass.

The protocols used here to investigate migration patterns (i.e., directionality) are following standard procedures, similar to those used to determine bird and insect directionality during migration. The protocols for birds are described in Emlen and Emlen (1966), Emlen and Emlen (1966) and have been widely used in birds and insects (Wiltschko et al., 1993, 2008; Able and Able, 1995; Benvenuti et al., 1996; Eng et al., 2017). Studies on amphibian migrations and orientation usually rely on setting such as drift-fence and pit-fall traps (Johnson, 2003; Todd et al., 2009; Santos et al., 2010; Lenhardt et al., 2015), emigration pattern field-data collection (Lenhardt et al., 2013), or indirectly through road-kills (Elzanowski et al., 2009). In addition, amphibian movements can also be studied through telemetry (Baldwin et al., 2006; Pellet et al., 2006; Borzée et al., 2018d) and orientation has also been studied through HDF tracking (Pašukonis et al., 2013; Pašukonis et al., 2014).

Tracking during brumation was conducted separately for the two treefrog species. HDF tracking was conducted in 2013 for *D. japonicus* because the species was already known to be hibernating on forested hills (Sugimoto and Jiang, 2008; Borzée et al., 2018c). However, we collected the first anecdotal observation on the overwintering of *D. suweonensis* in 2015, following which we conducted brumation tracking.

We released and tracked each frog for 24 h, thus including both diurnal and nocturnal activities. Releases were conducted after sunset, at least 15 m from each other to prevent detection overlap. For each tracking point, we placed a colored flag approximately 10 cm from the frog to measure the displacement and directionality of the movement between successive points. Every hour, we recorded temperature (°C), luminosity (lux), relative humidity (%), height from the ground (cm), distance moved from the previous point (cm) and type of microhabitat. The five types of microhabitats were "grass," "rice," "buried," "ground," and "bush." We also noted the directionality of the movement, toward either the center of the forest or the center of the adjacent rice paddies. The movement was decomposed in the form of a vector, for instance, 50 cm toward the forest and 20 cm toward the rice paddies. We took all measurements at 5-cm resolution to avoid overly disturbing the frogs.

Dryophytes japonicus

For this experiment, the waistband to attach the antenna to the frogs was made of gauze sewed onto itself ventrally and it was thus adjusted for the size of each individual. We tracked nine males and one female *D. japonicus* in the city of Paju (red marker; **Figure 1**), on September 27 to 30, 2013. As it is difficult to catch adults once they have reached their hibernation habitat, individuals had been previously caught in the forest adjacent to the rice paddy complex (see Borzée et al., 2018c) and raised for 1 month under controlled conditions in the lab. We released each individual at the edge of a rice paddy between 174 and 209 m away from the edge of the forest where it had been caught. We tracked individuals for 2328.33 ± 719.63 min on average (ca. 39 h), resulting in an average of 125.98 ± 17.70 min (mean ± S. D.; ca. 2 h) between tracking points, for a total of 172 observations.

Dryophytes suweonensis

For this experiment, each waistband was made of silicone tubing (diameter = 1.8 mm) within which we inserted the loop of the antenna. The loop was connected ventrally with electrical paint (BareConductive 10 ML, Bare Conductive Ltd; London, UK) and thus worked as an electric dipole that was tailored for the size of each individual. We tracked four individuals of each sex at four independent sites over a 110-km range (green markers, **Figure 1**) between October 8 and 16, 2015. Individuals had been caught ~1 month earlier at the same site (Kim, 2016; Borzée et al., 2018e) and had been raised in controlled laboratory conditions before their release. We released each individual at the edge of a rice paddy between 139 and 1,018 m away from the edge of the closest forest, and we tracked individuals for 1,332 ± 120 min on average (ca. 20 h), for a total of 305 observations.

Laboratory Brumation and Hibernation Observations

We conducted two experiments under laboratory conditions for both species, the first one on microhabitat selection during brumation, referred to as "lab brumation," and the second one on microhabitat selection during hibernation, referred to as "lab hibernation." For both species, the individuals originated from egg masses that we collected from the wild (Kim, 2016; Borzée et al., 2018e) and were kept in the lab from hatching to release (permit 2015-4 issued by the Ministry of Environment of the Republic of Korea). For this experiment, we collected five individuals from five egg masses at five different locations (blue markers, **Figure 1**; $n = 25$ for each species) and raised the tadpoles in independent PVC aquariums (20 cm W $\times$ 30 cm L $\times$ 20 cm H). After metamorphosis, individuals were isolated, providing a sample size of five individuals raised independently for each of the five families for each species. Each metamorph was raised in a glass terrarium 45 $\times$ 45 $\times$ 45 cm with a lateral opening, transparent sides, and a screen top (PT2605, Exo-terra, Hagen, Montreal, Canada).

We set all terraria with wet towels at the bottom that were changed weekly or more often if needed. We also set a conic non-glazed terra cotta pot (25 cm height $\times$ 16 cm diameter) horizontally at the back left of each terrarium and set a glazed water-dish (3 cm deep, 12 cm diameter) at the front right, with a 5 $\times$ 3 cm non-glazed terra-cotta cylinder pot set upside down within the water dish. The arrangement allowed for the terra cotta pots to absorb water and release it through evaporation within the terrarium to keep the humidity relatively constant (48.48 $\pm$ 11.18% rH). Finally, we set a wooden cylinder (2.5 cm diameter) from the bottom front left corner to the top back right corner. We used oak cylinders on *D. japonicus* known preferences (Borzée et al., 2018c). We set the terraria onto two four-layered shelves, with their position randomized every second week. Each terrarium was illuminated by its own lighting (UV-B bulbs), and we estimated that the positions of the terraria did not result in any bias in the experiments.

Each of the 50 terraria was sprayed daily and the water dish refilled *ad libitum* with carbon filtered and 72-h evaporated water. The frogs were set on a circadian cycle that matched the natural one, readjusted weekly, under natural spectrum illumination. Crickets were the main diet items used to feed the frogs, but fruit flies and maggot supplements were also given when available. All prey items were powdered with calcium and multivitamins prior to use.

For both brumation and hibernation experiments, we recorded the position of the individual, i.e., substrate use, and its height from the bottom of the terrarium three times a day (variable: "time period"). We also collected date, time, temperature (°C) and relative humidity (%) for each survey point. We noted temperature and humidity readings once for all 50 terrarium replicates because they were set in a common rearing room that was exposed to controlled climatic variations that followed natural variations (see Borzée et al., 2018e for details). There were five categories of microhabitat in each aquarium: big pot (on or within the large terra cotta pot, representative of sheltering behavior), ground (anywhere on the paper towel, representative of ground microhabitats), wood (sitting on the wood cylinder, representative of perching behavior on wooden microhabitats, as known to be important for *D. japonicus* brumation; Borzée et al., 2018c), pot in water (representative of flooded or damp habitat, as known to be important for *D. suweonensis* brumation, earlier in this study), and glass (when an individual was resting on a glass panel, not representative of any wild habitat but included to prevent any bias in further analysis). Whenever we made an observation, we noted the microhabitat the individual used.

We conducted the brumation experiment between September 23 and October 16, 2015, leading to a total of 1,906 observations, and we conducted the hibernation experiment between December 1 and 23, 2015, for a total of 1,612 observations.

Winter Field Observations

The purpose of this set of observations was to observe frogs of both species hibernating in their natural environments. We revisited the four sites where we had conducted brumation observations in the third week of January 2015 to look for buried individuals. We spent a total of 4 h at each of the four sites digging the soil down to 50 cm in the areas where the frogs of the two species had last been seen. The areas searched were about 2 m^2 for each observation, and matched with the areas where the frogs were last seen (Borzée and Jang, 2017), based on the assumption that home range and breeding ranges may be similar (Kim et al., in press).

Spring Orientation Tracking

For this section, the tracking methodology was the same as used for orientation experiments (Section Field Orientation Tracking for Brumation). The frogs used for this experiment were animals we had raised from hatching and had used for the two lab experiments. We had maintained them in the setting described above until the beginning of tracking experiments, and we released them after finishing the spring orientation tracking experiments. The sites for the experiment, which aimed at determining the post-hibernation behavior of the two treefrog species, were distributed along the whole range of *D. suweonensis* (blue markers, **Figure 1**). The sites were the same as the sites where we had collected the egg masses. The tracking procedure in the spring tracking experiment was the same as that of the fall tracking for *D. suweonensis*.

We conducted the fieldwork between May 25 and June 17, 2016, for a total of 20 individuals from each species. We released ten individuals of each species in rice paddies and the remaining ten in forested areas at the edge of rice paddies. The forest was composed of Chinese chestnuts (*Castanea* spp.) and pine trees (*Pinus* spp.) where *D. japonicus* is typically found during the non-breeding season (Borzée et al., 2018c). We checked the position of each individual every hour and we took note of date, time of day, GPS coordinates, vegetation type, temperature, humidity, height, total movement and movement toward rice paddies and forest. We assessed the directionality based on movements toward the selected landscape features. We spent an average of 22.07 $\pm$ 2.32 h ($n = 25$ for each species) tracking per individual (duration $\pm$ SD). As all individuals were tracked for over 16 h, all were included in

the subsequent analyses. The microhabitats recorded were grass, rice, buried, ground, and bush. We tracked a maximum of 5 individuals at the same time, and avoided tracking individuals within 50 m of each other to prevent overlap in HDF detection signal. We released each individual between 20:00 and 00:00 to minimize possible predation due to the tracking apparatus.

Data Analysis

After the tracking experiments, we mapped the GPS coordinates for all tracked points for each individual on Google Earth (Google, Mountain View, USA). For each data point, we then measured the directionality of the movement, such as the angle between the forest, the tracking position of the individual and its subsequent tracking position. Here, the forest was defined as a point situated at the center of gravity of 10 points randomly chosen on the edge of the forest. Because we had released all individuals at different points and their orientation toward landscape elements was our prime focus, and not directionality toward cardinal points, we defined 0 degrees as toward the forest. We set this point arbitrarily for ease of graphical interpretation and because individuals rarely expressed a total change (i.e., 180 degrees) in directionality. Thus, an angle of orientation between 270 and 90 degrees represented a displacement that factored positively toward the forest, an angle of 0 degrees was a straight line toward the forest and an angle of 180 degrees was a straight line away from the forest. We measured all angles on screen with the software imageJ (National Institutes of Health, Bethesda, USA). For ease of analysis for models assessing directionality of migration patterns but not involving interspecies comparisons, the directionality was binary encoded as toward or away from the forest. For other analyses where a binary encoding would not be adequate to describe the behavior of the species, variation in the angle of directionality was analyzed separately with circular statistics.

(1) To assess seasonal variations in occurrence for both species, and compare the occurrence patterns between the two species, we first analyzed the dataset through a repeated-measure ANOVA. To do so, the repeated surveys, continuous variable hereafter defined as "season," were set as the dependent variable with seven levels, corresponding to the seven surveys replicates, while occurrence for *D. suweonensis* and *D. japonicus* were set as factors. To run this analysis, we tested for homogeneity of variance with Levene's test, and because the error variance was not equal across groups for the sixth replicate only, we ignored the partial violation of assumption for the statistical analysis. We also tested the sphericity assumption with Mauchy's test, and ran the repeated-measures ANOVA with the Greenhouse-Geisser correction (Scheiner and Gurevitch, 2001) because the sphericity assumption was violated. Furthermore, we assumed compound symmetry (homogeneity of the variance-covariance matrix) for this analysis. We then graphically matched the variation in occurrence over weeks for the two species and the type of site with the results of the statistical analysis.

(2) Because there was no correspondence between the data for *D. japonicus* and *D. suweonensis*, the data for each species was first analyzed in relation with directionality to the

forest (details below), and then compared. The fall tracking data for *D. japonicus* was first tested for the significance of directionality toward forests for individuals. Because data were either temporally or spatially independent, the directionality variable was set as dependent variable in a binary logistic regression. The last assumption was also met in that the individual variable, set as the independent variables, was on a nominal scale.

Once directionality was established, we analyzed the dataset using a univariate General Linear Model (GLM) to find the factors that were important for directionality. Thus, we set directionality as a dependent variable, encoded as toward paddies, toward forests, or no movement; distance traveled as a fixed factor; frog ID, day, time of day and microhabitat as random factors and temperature and luminosity as covariates under a main effect model. After visually testing for the absence of outliers by analyzing box-plots, we determined the normal distribution of the data with the Kolmogorov-Smirnov test for normality with the Lilliefors significance correction ($0.14 \leq D_{(151)} \leq 0.51$, $p < 0.001$), and determined the homogeneity of variance with Levene's test for homogeneity of variances [$F_{(134,116)} = 2.19$, $p = 0.001$]. We did not include relative humidity in the analysis because it was correlated with temperature (Pearson's Correlation, $R = -0.16$, $p = 0.044$). We subsequently explained all significant variables through descriptive statistics.

We analyzed the fall tracking for *D. suweonensis* in a similar way as that for *D. japonicus,* first testing for the significance of directionality toward forests for individuals. Because the data were either temporally or spatially independent, we set directionality as the dependent variable in a binary logistic regression. The last assumption was also met in that "individual," set as the independent variable, was on a nominal scale.

We then analyzed our dataset with a GLM to find the factors that were important for directionality. For this model, we set directionality as the dependent variable; distance traveled as a fixed factor; site, sex, and vegetation as random factors and date, time of day, temperature, height, luminosity, and frog ID as covariates, under a main factor effect model. After visually testing for the absence of outliers by analyzing box-plots, we determined the normal distribution of the data with the Kolmogorov-Smirnov test for normality with the Lilliefors significance correction ($0.07 \leq D_{(301)} \leq 0.47$, $p \leq 0.001$), and determined the homogeneity of variance with Levene's test for homogeneity of variances [$F_{(136,164)} = 4.28$, $p < 0.001$]. In this analysis as well, we did not include relative humidity because it was correlated with temperature (Pearson's Correlation; $R = -0.81$, $n = 304$, $p < 0.001$). The significant variations were then explained through descriptive genetics.

Last, to understand the differences in movement patterns between the two species, we also analyzed the angles described by the movements in relation to forests using circular two-sample geometrical directional analysis. The data were temporally and spatially independent but did not meet the prerequisite assumption of the von Mises distribution (Watson's U2 test; *U2* $= 1.72$, $p < 0.005$; Lockhart and Stephens, 1985), and we used the non-parametric Mardia-Watson-Wheeler (Mardia, 1972) test with angle as the dependent variable and species as independent

variables. We ran the analysis under an axial (orientation) model. We conducted this additional test despite the experiments being conducted at different years and at different sites for the two species. We used the results to link the two analyses and highlight the differences between the two species.

(3) We first analyzed the lab brumation experiment dataset using a multinomial logistic regression to detect variation in microhabitat use between the two species. Thus, we set microhabitat as the dependent variable, and the independent variables were species, family, and individual ID nested within family as factors, and temperature, height, time and date as covariates. We did not use humidity in the model because it was correlated with temperature (Pearson's Correlation; $r = 0.40$, $p < 0.001$) and date ($r = 0.11$, $p = 0.001$). We ran the regression under a main-effects model and selected a multinomial logistic regression because assumptions were fulfilled: we did not detect outliers in our analyses of the box-plots. There was a linear relationship between the continuous independent variables and the logit transformation of the dependent variable, tested through the Box-Tidwell (Box and Tidwell, 1962) procedure with Bonferroni corrections (Tabachnick and Fidell, 2014), with $p > 0.379$ for all variables, which thus rejected the null-hypothesis. We then described the variation between the different microhabitat and other significant results.

We then analyzed the lab hibernation experiment dataset for microhabitat use in the same way as for the brumation period, because we conducted this experiment in the same setting and collected the type same data. We also ran the multinomial logistic regression in agreement with assumptions: we observed no outliers through the analysis of box-plots, and we did not use humidity in the model because it was correlated with temperature (Pearson's Correlation; $r = 0.09$, $p < 0.001$) and date ($r = 0.08$, $p = 0.001$). There was a linear relationship between the continuous independent variables and the logit transformation of the dependent variable with $p > 0.116$ for all variables.

As the same variables were significantly different for the two species for both phases of the experiment, as tested above, we proceeded to run an additional multinomial logistic regression to assess whether the two species differed in microhabitat use between the two phases of the experiment (i.e., brumation and hibernation). To do this, we set microhabitat as the dependent variable, and for the independent variables, we set phase and species as factors and height as a covariate. The model assumptions were met, we did not detect any outlier, no variables were significantly correlated and there was a linear relationship between the continuous independent variables and the logit transformation of the dependent variable with $p > 0.358$ for all variables.

(4) Due to the low number of hibernating individuals found during the field observations, we could not conduct any statistical analysis and the results are descriptive only.

(5) To determine microhabitat preferences and directionality post-hibernation for the two species, we first assessed the correlations between variables to avoid collinearity in subsequent analysis. We detected significant correlations between total displacement and displacement toward paddies (Pearson's

correlation; $r = 0.20$, $n = 873$, $p < 0.001$) and between total displacement and displacement toward forest (Pearson's Correlation; $r = -0.26$, $n = 873$, $p < 0.001$). The variables ID, date (Pearson's Correlation; $r = 0.95$, $n = 873$, $p < 0.001$) and sites (Pearson's Correlation; $r = 0.96$, $n = 873$, $p < 0.001$) were also correlated. Finally, temperature and humidity followed the same trend (Pearson's Correlation; $r = -0.79$, $n = 873$, $p < 0.001$). Among the correlated variables, we included only one from each group in the subsequent analyses.

We then ran a GLM to determine the differences in directionality between the two species post-hibernation. Thus, the binary encoded directionality was set as the dependent variable, species and ID as fixed factors, habitat as a random factor, and date, time, temperature and height as covariates, under a main-effects model. A few more variables were not included in the model because of collinearity: humidity was correlated with temperature (Pearson's Correlation; $r = -0.78$; $n = 873$; $p < 0.001$), total displacement was correlated with directionality (Pearson's Correlation; $r = 0.33$; $n = 873$; $p < 0.001$), and site type with microhabitat (Pearson's Correlation; $r = -0.41$; $n = 873$; $p < 0.001$). We chose a GLM after visually testing for the absence of outliers through the analysis of the box plots, and we determined the normal distribution of the data with the Kolmogorov-Smirnov test for normality with the Lilliefors significance correction ($0.14 \leq D_{(151)} \leq 0.51$, $p < 0.001$). Finally, we determined the homogeneity of variance with Levene's test for homogeneity of variances [$F_{(68,804)} = 6.03$, $p = 0.001$]. The significant differences were highlighted by descriptive statistics, and in addition T-tests, circular statistic tests and *ad hoc* analyses were conducted if required. Due to significant differences in directionality between the two species, we analyzed the differences in displacement angles through a suite of circular two-sample geometrical directional analyses, two-by-two for the variables: species, release habitat and angles. Because the data were temporally and spatially independent, but did not meet the prerequisite assumption of von Mises distribution (Watson's U2 test; U2 = 2.65, $p < 0.005$), we used the non-parametric Mardia-Watson-Wheeler test (Mardia, 1972) with angle as a dependent variables and either species or release habitat as the independent variable. We ran the analyses under an axial (orientation) model. Specifically, we computed the biostatistical analyses using SPSS v 21.0 (IBM Corp., Armonk, USA) while the circular statistics were conducted under PAST v 3.17 (Hammer et al., 2001).

RESULTS

Brumation Field Observations

The field surveys during the brumation period highlighted a clear difference in habitat preference between the two treefrog species: during brumation, *D. suweonensis* was present at rice paddies only while *D. japonicus* occurred at both rice paddies and forests (**Figure 3**). However, *D. japonicus* was present in rice paddies until the last week of September only, after which the species was only seen in forests. Neither species was detectable by spotlight transects from the last week of October, temporally matching with the first freeze. The results of the repeated-measure ANOVA
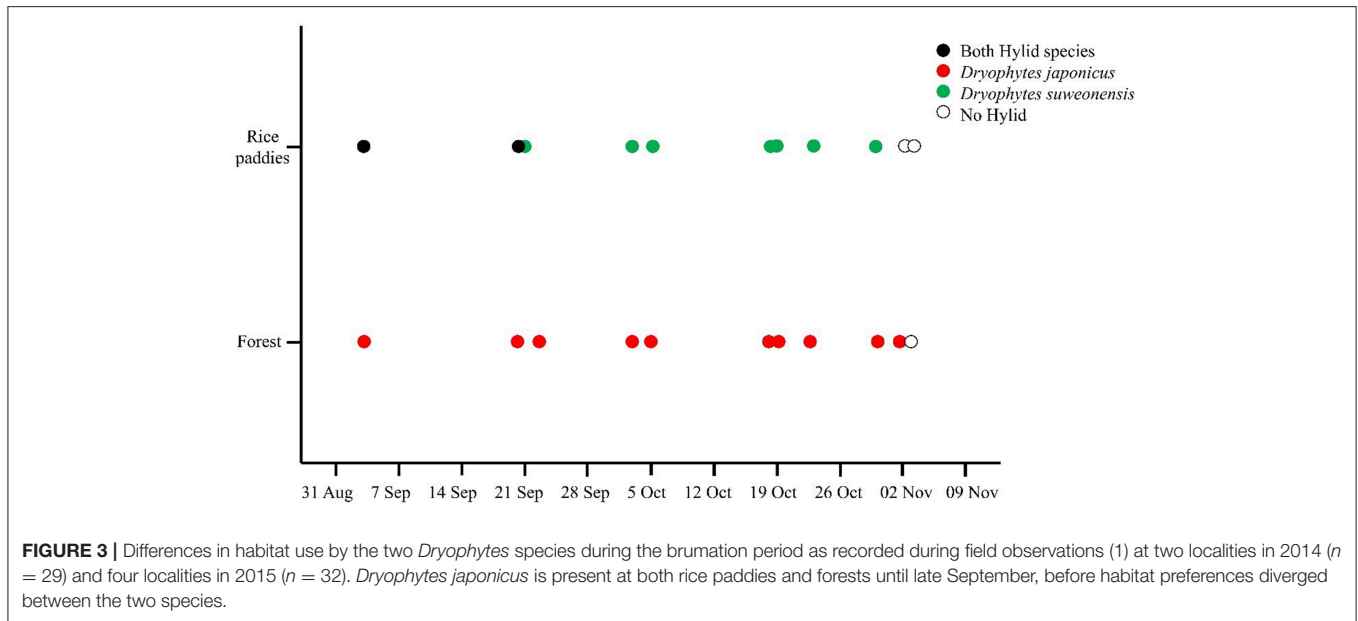
FIGURE 3 | Differences in habitat use by the two *Dryophytes* species during the brumation period as recorded during field observations (1) at two localities in 2014 (*n* = 29) and four localities in 2015 (*n* = 32). *Dryophytes japonicus* is present at both rice paddies and forests until late September, before habitat preferences diverged between the two species.

(*n* = 61) supported these results, showing that the occurrence of *D. suweonensis* significantly varied with season ($\chi^2 = 0.64$, $d = 6$, $p < 0.001$), and *D. japonicus* followed the same pattern ($\chi^2 = 0.58$, $d = 6$, $p < 0.001$). Thus, our results highlight a significant variation in habitat use: both species were present in rice paddies at the beginning of the surveys, whereas *D. suweonensis* was in the rice paddies and *D. japonicus* was in the forests only during the week preceding hibernation.

Field Orientation Tracking for Brumation

During the fall tracking (2) experiment for *D. japonicus* in 2013, there was a clear directionality pattern. The species moved away from the rice paddies in 63.6% of cases (*n* = 96), and toward the forest in 66.2% of cases (*n* = 100), while moving toward paddies in 36.4% of cases (*n* = 55), and away from forests in 33.8% of cases (*n* = 51). Here, away from paddy and toward forest conveys the same directionality vector, but the percentages do not equal 100% if combined two by two because the directionality is divided into vectors that can have multiple constituents. The logistic regression model was statistically significant, $\chi^2_{(1)} = 8.38$, $p = 0.004$ and the model explained 78.0% (Nagelkerke pseudo-R^2) of the variance in directionality and correctly classified 58.9% of cases.
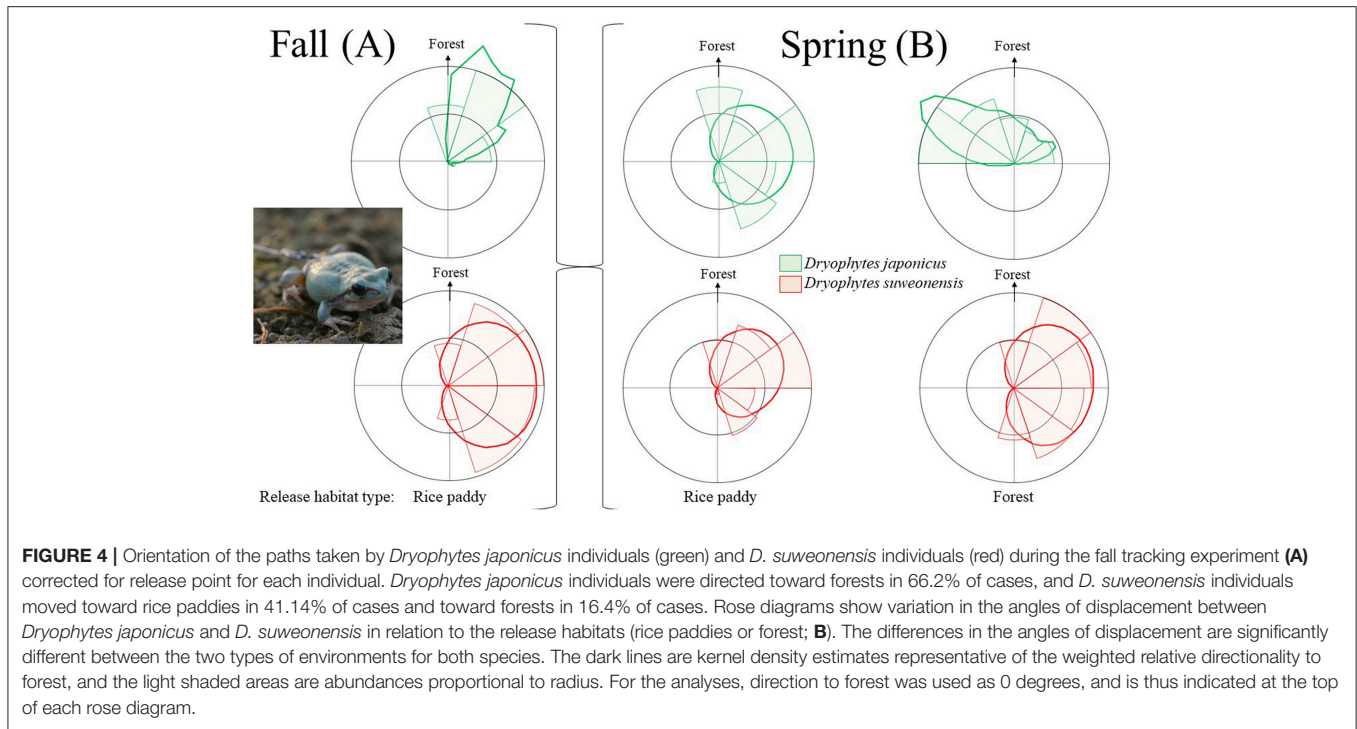
Dryophytes japonicus used the grass and bush microhabitats to move toward paddies in 1.8% of cases (*n* = 3) and while they used the rice microhabitat in 96.4% of cases (*n* = 146). Most movements toward forests were also made in rice (94%; *n* = 142), while remaining movements toward forest were in grass (4%; *n* = 6) and bush (2%; *n* = 3). The distances traveled in relation to directionality were also significantly different between the two species (**Table 1**; **Figure 4**), with an average distance moved toward the rice paddies of 48.10 ± 7.8 cm and an average distance moved toward the forest of 99.4 ± 11.2 cm. ID, day,

TABLE 1 | GLM to test the relationships between directionality and other factors collected during the tracking experiment to investigate the brumation behavior of *Dryophytes japonicus*.

		Type III-$\Sigma\chi^2$	df	χ^2	F	p-value
Distance traveled	Hypothesis	13.95	24	0.58	30.47	0.032
	Error	0.04	2	0.02		
Individual ID	Hypothesis	1.84	8	0.23	12.09	0.079
	Error	0.04	2	0.02		
Day	Hypothesis	0.52	2	0.26	13.52	0.069
	Error	0.04	2	0.02		
Time	Hypothesis	16.90	100	0.17	8.86	0.107
	Error	0.04	2	0.02		
Habitat	Hypothesis	0.39	1	0.39	20.69	0.045
	Error	0.04	2	0.02		
Temperature	Hypothesis	0.03	1	0.03	1.83	0.309
	Error	0.04	2	0.02		
Luminosity	Hypothesis	0.01	1	0.01	0.48	0.559
	Error	0.04	2	0.02		

time of day, temperature, and luminosity were not significant to directionality (**Table 1**).

During the fall tracking experiment (2) for *D. suweonensis* in 2015, there was no clear difference in directionality, in contrast with *D. japonicus* (**Figure 4**). The species moved away from the rice paddies in 3.0% of cases (*n* = 9) but toward paddies in 41.14% of cases (*n* = 125), and moved away from forests in 20.4% of cases (*n* = 62) and toward forests in 16.4% of cases (*n* = 50). The percentages do not equal 100% if combined two by two because the directionality is divided into vectors that can have multiple constituents. Once tested statistically, the logistic regression model was not significant, $\chi^2_{(1)} = 0.56$,

FIGURE 4 | Orientation of the paths taken by *Dryophytes japonicus* individuals (green) and *D. suweonensis* individuals (red) during the fall tracking experiment **(A)** corrected for release point for each individual. *Dryophytes japonicus* individuals were directed toward forests in 66.2% of cases, and *D. suweonensis* individuals moved toward rice paddies in 41.14% of cases and toward forests in 16.4% of cases. Rose diagrams show variation in the angles of displacement between *Dryophytes japonicus* and *D. suweonensis* in relation to the release habitats (rice paddies or forest; **B**). The differences in the angles of displacement are significantly different between the two types of environments for both species. The dark lines are kernel density estimates representative of the weighted relative directionality to forest, and the light shaded areas are abundances proportional to radius. For the analyses, direction to forest was used as 0 degrees, and is thus indicated at the top of each rose diagram.

$p = 0.453$, explaining 0.1% (Nagelkerke pseudo-R^2) of the variance in directionality and correctly classified 55.4% of cases. There was thus a difference between the two species, with *D. japonicus* displaying a significant preferential direction whereas *D. suweonensis* did not.

The results of the GLM explaining the factors related to directionality show that only the distance traveled was significant (**Table 2**). Despite the different distances to forest at the four sites (139, 1018, 470, and 404 m), no significant variation among sites were reported, and it was the same for the variation between individuals, highlighting a general behavior (**Table 2**). The average distance moved toward the rice paddies between two locations was 78.75 ± 235.98 cm while the distance traveled toward the forest was on average -38.18 ± 219.34.

We found a difference in directionality through angles of displacement for the two species in that the angle described by the movements of *D. japonicus* deviated from the forest by 34.79 ± 5.09 (mean $\pm$ kappa) degrees only on average, whereas the angle described by *D. suweonensis* was 87.55 ± 1.79 degrees away from the forest on average (**Figure 4**). We found the difference in directionality between the two species to be significant (Mardia-Watson-Wheeler test; $W = 461.80$, $n = 288$, $p < 0.001$).

Laboratory Brumation and Hibernation Observations

Brumation

During the brumation period, we observed a significant difference in microhabitat use between the two species (**Table 3**) under a significant model ($\chi^2 = 3266$, $df = 212$, $p < 0.001$), explaining 85.9% of variation (Nagelkerke

TABLE 2 | GLM to test the relationships between directionality and other factors collected during the tracking experiment to investigate the brumation behavior of *Dryophytes suweonensis*.

		Type III- $\Sigma \chi^2$	df	χ^2	F	P-value
Distance traveled	Hypothesis	115.53	75	1.54	9.53	<0.001
	Error	34.42	213	0.16		
Site	Hypothesis	0.39	2	0.19	1.19	0.306
	Error	34.42	213	0.16		
Sex	Hypothesis	0.02	1	0.02	0.15	0.700
	Error	34.42	213	0.16		
Vegetation	Hypothesis	0.61	3	0.20	1.27	0.286
	Error	34.42	213	0.16		
Date	Hypothesis	0.29	1	0.29	1.80	0.181
	Error	34.42	213	0.16		
Time	Hypothesis	0.21	1	0.21	1.29	0.258
	Error	34.42	213	0.16		
Temperature	Hypothesis	0.18	1	0.18	1.14	0.286
	Error	34.42	213	0.16		
Height	Hypothesis	0.02	1	0.02	0.10	0.750
	Error	34.42	213	0.16		
Lux	Hypothesis	0.03	1	0.03	0.18	0.668
	Error	34.42	213	0.16		
Individual ID	Hypothesis	0.05	1	0.05	0.33	0.568
	Error	34.42	213	0.16		

pseudo-R^2). *Dryophytes japonicus* preferentially selected the wood microhabitat (20.0% use in *D. japonicus* and 10.0% in *D. suweonensis*), whereas *D. suweonensis* preferentially selected the ground microhabitat (24.0% use in *D. suweonensis* and 12.6% use in *D. japonicus*; **Figure 5**). These microhabitats were the most

commonly used ones after removing the "glass" microhabitat used for displacements (37% in *D. japonicus* and 34.0% in *D. suweonensis*).

One of the other significant differences between the two species was the height at which the frogs were found in the terraria (**Table 3**). *Dryophytes japonicus* was on average 23.14 ± 4.33 cm high, whereas *D. suweonensis* was on average 20.37 ± 7.37 cm high. In addition, there was a significant difference between individuals and families, and the model for this analysis was significant ($\chi^2 = 373.48$, $F = 264.41$, $p < 0.001$).

Hibernation
During the hibernation period, the variables microhabitat and height were also significantly different between the two species (**Table 4**), under a significant model ($\chi^2 = 2138.21$, $df = 232$, $p < 0.001$) that explained 79.1% of the variation (Nagelkerke

	χ^2	*df*	*P*-value
Species	30.53	16	<0.001
Family	59.45	16	<0.001
ID(Family)	514.65	176	<0.001
Temperature	13.42	4	0.009
Height	2260.70	4	<0.001
Time	5.62	4	0.229
Date	44.35	4	<0.001

pseudo-R^2). In this second phase of the experiment, the use of the flooded microhabitat was higher for *D. japonicus* (5.7%) than for *D. suweonensis* (1.7%) while *D. japonicus* moved about twice more than *D. suweonensis*, as seen by the greater use of glass walls (12.9 vs. 6.9%; **Figure 5**).

According to the results of the brumation experiment, the heights at which individuals were found was also significantly different (**Table 4**), with *D. japonicus* again higher on average (7.19 ± 9.47 cm) than *D. suweonensis* (5.21 ± 8.10 cm). Besides, there was a significant difference between families. The model for this analysis was also significant ($\chi^2 = 463.33$, $F = 663.08$, *p*-value < 0.001).

Difference Between Brumation and Hibernation
The multinomial logistic regression to assess whether the two species differed in microhabitat use between brumation and hibernation were significant for species ($\chi^2 = 44.83$, $df = 4$, $p < 0.001$), phase ($\chi^2 = 118.27$, $df = 4$, $p < 0.001$) and height ($\chi^2 = 4362.12$, $df = 4$, $p < 0.001$). The model was significant ($\chi^2 = 5069.81$, $df = 12$, $p < 0.001$) and explained 78.9% of the variance (Nagelkerke preudo-R^2). As seen earlier, the frequency of use for the wood microhabitat decreased between the two species between brumation and hibernation, while the frequency increased for the use of the ground microhabitat (**Figure 5**). The average height also decreased for the two species during the same period.

Winter Field Observations
Out of the 4 h spent digging at each of the sites, we found a single individual, a female *D. suweonensis* at the paddy site in Sihung (site 1; 37.410046°N; 126.808053°E; **Figure 2**). We found

FIGURE 5 | Microhabitat variation displayed by *Dryophytes suweonensis* and *D. japonicus* during brumation and hibernation with corresponding frequency. The variation in the total number of counts was due to the death of three individual *D. suweonensis* during the period before hibernations. The non-annotated stack "pot in water" for *D. suweonensis* is 1.7%.
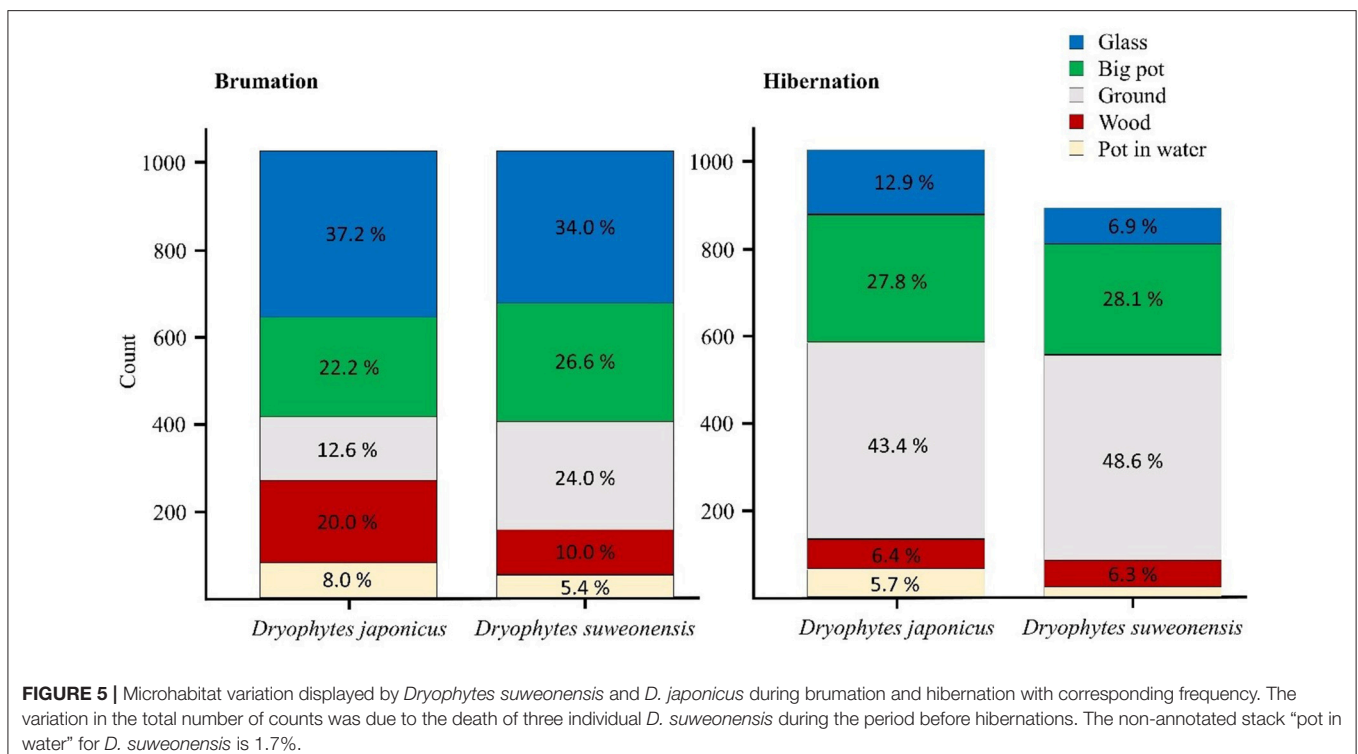
TABLE 4 | Results of the multinomial logistic regression to investigate variation in microhabitat use between the two species during the hibernation experiment ($n = 1,750$).

	χ^2	df	P-value
Species	34.06	4	<0.001
Family	72.05	16	<0.001
ID (Family)	258.58	196	0.002
Temperature	16.80	4	0.110
Height	1738.50	4	<0.001
Time	2.72	4	0.606
Date	3.51	4	0.045

TABLE 5 | Results of the GLM to test the factors of importance between the two species during the spring tracking experiment.

	Type III-$\Sigma\chi^2$	df	χ^2	F	P-value
Species	1.14	1	1.14	4.25	0.040
ID	7.22	24	0.30	1.12	0.309
Habitat	5.66	4	1.41	5.29	0.000
Date	0.28	1	0.28	1.06	0.304
Time	0.11	1	0.11	0.40	0.527
Temp	0.07	1	0.07	0.26	0.610
Height	0.04	1	0.04	0.16	0.686
Error	224.48	839	0.27		

TABLE 6 | Descriptive statistics for the directionality of the movements exhibited by *Dryophytes suweonensis* and *D. japonicus* in relation to the type of site selected for the release (i.e., rice paddy or forest) during the spring tracking experiments.

Habitat	Species	
Forest	*D. japonicus*	*D. suweonensis*
DIRECTIONALITY IN RELATION TO FOREST		
Away from forest	60.1	56.7
Toward forest	13.9	43.3
DIRECTIONALITY IN RELATION TO PADDY		
Away from paddy	15.9	22.4
Toward paddy	46.2	77.6
Paddy		
DIRECTIONALITY IN RELATION TO FOREST		
Away from forest	50.9	45.5
Toward forest	49.1	54.5
DIRECTIONALITY IN RELATION TO PADDY		
Away from paddy	40.4	40.0
Toward paddy	59.6	60.0

Data pooled two-by-two do not equal 100% because frogs were sometimes immobile and thus did not provide any directionality data for a few hours.

the individual in a burrow excavated by an unknown animal. The individual was buried between 27 and 30 cm deep. The absence of other findings does not reflect the absence of individuals, but only our inability to find them.

Spring Orientation Tracking

The directionality exhibited by *D. japonicus* and *D. suweonensis* during the spring tracking experiment was significantly different between the two species (**Table 5**). When released in the forest habitat, 60.1% of *D. japonicus* displacements were away from the forest release point, and 42.6% toward rice paddies (**Table 6**), a significant difference between the two directions for the species (*T*-test; $t = -8.15$, $df = 127$, $p < 0.001$). In contrast, there was no difference in directionality between the two species when released in the rice paddies (**Table 6**; *T*-test; $t = -1.31$, $df = 45$, $p = 0.198$). Oppositely, the movements of *D. suweonensis* toward and away from the forest when released in that habitat were not significantly different (**Table 6**; *T*-test; $t = -3.49$, $df = 50$, $p = 0.186$), neither than it was significant when released in rice paddies (**Table 6**; T-test; $t = -0.32$, $df = 46$, $p = 0.749$; **Figure 4**). This pattern was the same for the cumulated distances traveled by the two species toward either rice paddies (*D. japonicus* = 20.60 ± 128.62 and *D. suweonensis* = 15.82 ± 119.01 m; ANOVA; χ^2 = 402.09, $F_{(1,872)} = 0.07$, $p = 0.786$) or forests [*D. japonicus* = −30.46 ± 119.36 and *D. suweonensis* 0.98 ± 115.80; ANOVA; χ^2 = 31439.84, $F_{(1,872)} = 6.61$, $p = 0.010$; here cumulated by type of site for ease of understanding].

FIGURE 6 | Variations in habitat use between *Dryophytes suweonensis* and *D. japonicus* during the spring tracking experiments. *Dryophytes japonicus* was not found buried in this study.

The results of the first GLM also showed a difference in microhabitat use between the two species (**Table 5**), with grass preferentially used by *D. japonicus*, and grass and bush principally used by *D. suweonensis*. However, we never found *D. japonicus* buried, and we found *D. suweonensis* buried in only 3.3% of cases and on the bare ground in only 6.3% of cases (**Figure 6**).

When assessing the difference in directionality for the two species (**Figure 7**), the angles were significantly different (Mardia-Watson-Wheeler tests) when released in rice paddies [*D. japonicus*: 79.22 ± 1.69 (mean ± kappa); *D. suweonensis*: 68.02 ± 2.00], and forests (*D. japonicus*: 113.77 ± 1.87;

D. suweonensis: 86.39 ± 1.57). In addition, the variables were significantly different (Mardia-Watson-Wheeler tests) for: habitat of release for both *D. japonicus* ($W = 364.76$, $n = 225$, $p < 0.001$) and *D. suweonensis* ($W = 326.79$, $n = 221$, $p < 0.001$) but also between both species for a given release habitat: rice paddies ($W = 306.23$, $n = 192$, $p < 0.001$) and forests ($W = 387.23$, $n = 245$, $p < 0.001$).

DISCUSSION

Extensively different traits can occur with little genetic change (West-Eberhard, 2005), and here we further developed the suspected divergence in evolutionary history between the two Korean species of *Dryophytes* treefrogs (Ham, 2014; Kim, 2016). Whereas, *D. japonicus* migrates twice yearly between rice paddies and forested areas, to breed and overwinter, *D. suweonensis* is present at rice paddies all year round and hibernates buried underground. The ancestors of the two species diverged during the Late Miocene (8.7∼6.5 Mya Duellman et al., 2016; Dufresnes et al., 2016), apparently when one of the two species adapted to a different environment. It is likely that this happened when *D. suweonensis* preferentially selected marshes for breeding, whereas the preference of the ancestral species is expected to have been broader, a characteristic shared by most *Hyla* and *Dryophytes* species. Breeding in a different habitat led to the acquisition of new behavioral traits, such as holding on vegetation while calling (Borzée et al., 2016a), but also to the loss of traits, here migration. It is unlikely that the seasonal migration is a newly acquired trait in *D. japonicus* given that the species breeds in most types of habitats, also at higher elevations (Roh et al., 2014), and thus migration is expected to have been the ancestral character. The migration distance may, however, have been modified by the development of agriculture, with the two species brought back into contact (Borzée et al., 2015b, 2017a). *Dryophytes suweonensis* breeding in a separate habitat is the preferred hypothesis as this scenario enables the development of pre-zygotic isolating traits, such as seen in *Dryophytes cinereus* (Höbel and Gerhardt, 2003), and the two species are able to hybridize (Kuramoto, 1984; Borzée et al., 2015b), and have thus not evolved post-zygotic isolation. Sympatric speciation is hypothesized as peri- and para-patric speciations cannot have occurred with the two species displaying sympatric ranges (Jang et al., 2011).

During the breeding season, both species call from rice paddies, and although calling is discontinued in early July (Roh et al., 2014), both species are present in the vicinity of rice paddies until mid-September, where individuals sometimes produce calls. Continued vocalizations are unlikely to be for mating purposes because juveniles attempt the same behavior in synchrony (Pers. Obs.). At this point in time, adult *D. japonicus* migrate toward forests, up to several hundred meters away in this study, although closely related species can migrate up to 8 km (Angelone and Holderegger, 2009). Males *D. japonicus* will be present on the tree canopy, favoring oak trees (Borzée et al., 2018c) and producing calls as observed during transects here, until they are not seen anymore, in late October/early November, temporally coinciding with the first frost. Adult and juvenile *D. suweonensis* stay in
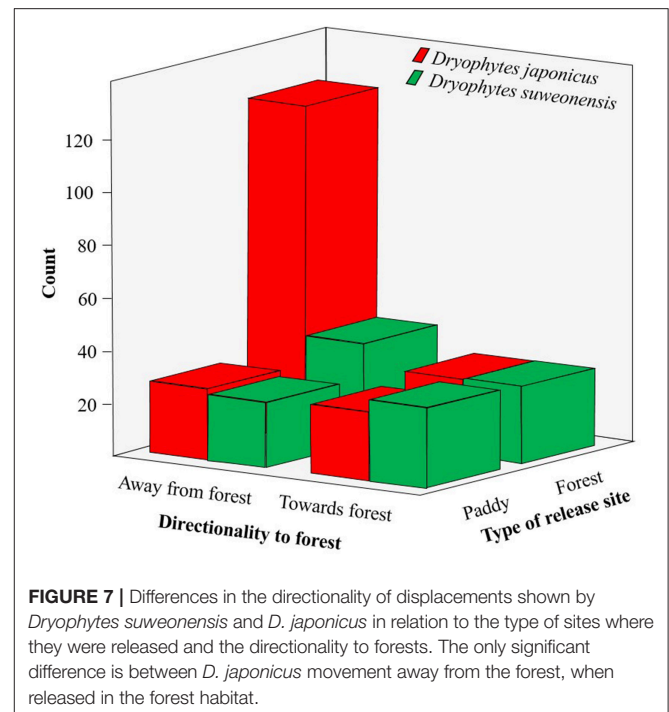

FIGURE 7 | Differences in the directionality of displacements shown by *Dryophytes suweonensis* and *D. japonicus* in relation to the type of sites where they were released and the directionality to forests. The only significant difference is between *D. japonicus* movement away from the forest, when released in the forest habitat.

the vicinity of rice paddies, favoring the upper leaves of planted beans (Borzée and Jang, 2017), hypothetically for feeding based on the high insect density seen in proximity. This, however, also makes the species susceptible to the bean harvest, which potentially affects recruiting young individuals into the breeding pool (Borzée and Jang, 2017). Individuals will then find shelter underground on the banks of rice paddies, where rice straw is stacked after harvest but also burnt before the thaw of ice, with unknown consequences for the species.

Both before and after overwintering, the two species displayed variations in the orientation of their displacements in relation to forests and rice paddies. *Dryophytes japonicus* was aiming at forested hills before winter, whereas *D. suweonensis* displayed non-directional displacements. After winter, the difference between the species was still present, with *D. japonicus* moving toward the breeding sites when released in the forest, whereas *D. suweonensis* did not display any directionality. It would seem that *D. suweonensis* was unable to find its way toward the breeding sites. When released in rice paddies, both of the species displayed non-directional displacements, an indication that they had reached their target sites, for breeding and feeding (Kim, 2015a), and that the directionality *D. japonicus* displayed toward forests before hibernation and toward rice paddies afterwards was not an artifact. Our results raise an interesting question regarding the methods used to display directionality. Amphibian species are known to rely on a set of methods to orientate their movement (see review by (Sinsch, 1990)). However, because all individuals had been kept in laboratory for a month prior to release, or were laboratory born, landscape and field recognition could not be learned. Also, rainy conditions during the fall orientation experiment reduced the possibility of celestial navigation or the use of polarized light.

The behavior expressed by the two species while they were kept in terraria during fall and winter differed clearly between species and between seasons following what would have been expected from wild individuals. During brumation, *D. suweonensis* was found on the ground of the terraria, whereas we found *D. japonicus* more often on wooden structures, at higher heights from the ground. The difference in height matches the brumating behavior we recorded in the wild, with *D. suweonensis* brumating in the vicinity of rice paddies, that is, in areas without high vertical structures, whereas *D. japonicus* is found on trees most of the time. The preference for bean leaves by *D. suweonensis* in the wild, around 50 cm high, may thus be more closely related to prey availability than microhabitat preference. The hibernation period saw the two species exploiting the ground microhabitat, and thus hints at the use of buried hibernacula for hibernating by the two species. *Dryophytes japonicus* was more active and was found higher up during the period, in agreement with the high freeze tolerance shown by the species (Storey and Storey, 2017).

Amphibian populations have greatly declined in recent decades (Blaustein et al., 1994), with approximately one third of all species currently under threat of extinction and more than 200 species already extinct (Hayes et al., 2002). The potential for extinctions in pristine environments such as Madagascar (Kolby, 2014) is still high, and basic ecological knowledge is still required for a high number of amphibians, as highlighted by the Data Deficient designation of species by the IUCN (International Union for Conservation of Nature) on its Red List of endangered species. In response to these losses, biodiversity conservation efforts have been deployed to tackle population decreases and extinctions (Marsh and Trenham, 2001; Gascon, 2007). However, and especially with amphibians, most conservation work is addressed to the breeding habitat, and thus, not all conservation efforts have been successful (Blaustein and Kiesecker, 2002). Failure is not necessarily attributable to the work carried out but because of limiting factors such as basic knowledge. We therefore urge the implementation of conservation measures for *D. suweonensis*, listed as endangered by the IUCN (IUCN,

2018) and under Korean law (Ministry of Environment, 2012), highlighting that protecting the totality of the space used by the species is easier than for most species, as hibernation and breeding habitats overlap. Despite the areas to protect being only in rice paddies, international conservation plans such as the ones developed under the Ramsar convention will meet the dual objective.

AUTHOR CONTRIBUTIONS

AB designed the experiment, collected and analyzed the data and wrote the manuscript. YC analyzed data and participated in writing. YK collected data and participated in writing. PJ supervised. YJ designed the experiment, revised the manuscript, and supervised the project.

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