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Cutaneous Resistance to Evaporative Water Loss in Taiwanese Arboreal Rhacophorid Frogs

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Jian-Nan Liu and Ping-Chun Lucy Hou (2012) Cutaneous resistance to evaporative water loss in Taiwanese arboreal rhacophorid frogs. *Zoological Studies* **51**(7): 988-995. In this study, we investigated whether arboreal rhacophorid amphibians in humid subtropical Taiwan exhibit significant cutaneous resistance (R_c) to evaporative water loss (CWL). Animals from 7 arboreal rhacophorid and 3 non-arboreal amphibian species were injected with a neuromuscular blocking agent to eliminate pulmonary water loss, and their rates of CWL were measured gravimetrically in a wind tunnel at a wind speed of approximately 1.0 m/s. For each individual, we used a same-sized 3% agar replica to determine its boundary layer resistance, which allowed us to calculate R_c. Our results showed that all 7 arboreal species exhibited a detectable R_c that ranged 1.2-3.3 s/cm, and rates of CWL of arboreal species were 1/2-1/5 those of non-arboreal species. We further tested whether arboreal species actively adjusted their R_c in response to an increase in CWL associated with a high wind speed. Rates of total evaporative water loss (EWL) and R_c of physically restricted animals were measured at wind speeds of 0.6 cm/s and 1.0 m/s. Results showed that the R_c of arboreal rhacophorid species did not significantly differ between the 2 wind speeds despite substantially increased rates of EWL at the high wind speed. A comparison between the CWL of paralyzed frogs and the EWL of restricted frogs showed that the ratio of pulmonary water loss to total EWL in Taiwanese amphibians is very low and can be neglected.
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Most aquatic, semi-aquatic, and terrestrial anuran amphibians have skin that is highly permeable to water. Their cutaneous resistance (R_c) to evaporative water loss (CWL) is very low or indistinguishable from a free water surface (Spotila and Berman 1976, Withers et al. 1984, Wygoda 1984, Prates and Navas 2009, Wygoda et al. 2011). These species rely to a large extent on behavioral avoidance of desiccation and search for water sources, and have been called "typical" amphibians (Wygoda 1984, Shoemaker et al. 1992). Several dozen arboreal amphibian species from the families Hylidae, Rhacophoridae, and Hyperoliidae, however, were reported to have significant R_c values (reviewed by Lillywhite 2006). Those species are considered "atypical" with regard to CWL (Wygoda 1984). R_c values of atypical amphibians vary among species, ranging from slightly waterproof (R_c = 1-10 s/cm) in many North American and Australian hylids, to moderately waterproof (10-100 s/cm) in several Australian *Litoria* and waterproof (200-400 s/cm) in some xerophilic African and South American frogs (Lillywhite 2006).

For atypical amphibian species, the rate of CWL is determined by 2 resistances, R_c and the boundary layer resistance (R_b), and a driving force resulting from the difference in water vapor

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density within the skin of the animal and in the free air outside the boundary layer surrounding that animal (Spotila and Berman 1976). Studies showed that R_b and CWL are significantly influenced by environmental factors such as wind speed (Bentley and Yorio 1979, Shoemaker et al. 1987). Little is known, however, about the capability of atypical amphibians to rapidly regulate their R_c in response to changing environmental conditions. Wygoda (1988) reported that *Hyla cinerea* actively increased its R_c from 1.7 to 3.7 s/cm when the ambient water vapor density decreased from 20.13×10^{-6} to 9.30×10^{-6} g/cm. He suggested that the increased R_c at low water vapor densities was because frogs increased skin mucus secretions with layers of dried mucus covering the skin which served as a water barrier. The environment of arboreal species is generally considered to have less accessibility to free water and have stronger air movements compared to ground-dwelling amphibians (Yorio and Bentley 1977, Shoemaker et al. 1987). Information of whether atypical arboreal amphibians increase their R_c in response to a rise in CWL associated with wind speed, however, is scarce.

The Rhacophoridae is the 2nd largest family of anuran amphibians in which members are primarily arboreal, consisting of 321 species across a wide range of tropical Africa and southern to eastern Asia (Frost 2011). In spite of a large body of literature on R_c value of anuran amphibians (Lillywhite 2006), there is comparatively insufficient knowledge of R_c values in rhacophorids, especially for species inhabiting wet regions. Taiwan is a subtropical island with relatively high average annual precipitation (~2500 mm) and relative humidity (~80%). Seasonal monsoons, however, often bring strong winds and result in an inhospitable situation for arboreal amphibian species with regard to CWL. Thus, rhacophorid amphibians in Taiwan are useful model species to address whether arboreal rhacophorid species in humid regions exhibit comparably high R_c values, and, if so, whether these species adjust their R_c in response to wind speed.

The 1st goal of this study was to examine whether Taiwanese arboreal rhacophorids exhibit significant R_c values. We predicted that Taiwanese arboreal rhacophorid frogs would exhibit R_c values of about 1-5 cm/s, similar to some arboreal hylid species inhabiting mesic or humid areas. We further tested the hypothesis that Taiwanese rhacophorids will increase their R_c when they experience an increase in the rate of CWL associated with increased wind speeds. Additionally, we examined the ratio of pulmonary water loss to total EWL in Taiwanese amphibians.

MATERIALS AND METHODS

Animals

Adult animals of 7 arboreal rhacophorid species, *Rhacophorus taipeianus*, *Rha. moltrechti*, *Rha. arvalis*, *Rha. prasinatus*, *Polypedates megacephalus*, *Kurixalus eiffingeri*, and *Buergeria robusta*, and 3 non-arboreal species, *B. japonica* (Rhacophoridae), *Duttaphrynus melanostictus* (Bufonidae), and *Rana longicrus* (Ranidae), were collected from 11 localities (all at elevations of < 500 m) across Taiwan. We considered *B. robusta* to be an arboreal species in the present study, although this species is often found on the ground. With the exception of *Rha. taipeianus* and *R. longicrus* that were collected and measured in Jan. 1996, all experiments were carried out between July and Sept. 1995. Collected animals were transported to National Cheng Kung Univ. (Tainan, Taiwan), where they were housed in plastic containers containing a shallow layer of water. Animals were acclimated at 21.0 ± 1.0°C, 75% ± 5% relative humidity (RH), and a 12-h light: 12-h dark photoperiod. The temperature was below the summer average temperature but was chosen by considering 2 species that are active during winter. Animals were fed mealworm larvae (*Tenebrio molitor*) by hand during the period of acclimation, but were starved for 3 d prior to measurements of water loss. During the period of captivity (all ≤ 10 d), all animals appeared in very good health, and their body mass did not change by > 5%. After the measurements were completed, the frogs were released into their original habitats.

Experimental arrangement

Two groups of frogs were used in 2 separate experiments. The 1st group of frogs (paralyzed frogs) was injected in the thigh muscle with tubocurarine chloride (Sigma T2379: 15-20 μg/g body mass for *D. melanosticus* and 3-7 μg/g body mass for other species; St. Louis, MO, USA) to eliminate activity and pulmonary water loss (Wygoda 1981). Rates of CWL of paralyzed frogs were measured at 21.0 ± 1.0°C and a wind speed of 1.0 ± 0.1 m/s. This wind speed removes most of the boundary layer (Foley and Spotila 1978) and was used in numerous studies (Wygoda 1984). All paralyzed frogs recovered a few hours after the experiments.

The 2nd group of frogs was used to test the hypothesis that arboreal frogs elevate their R_c in response to a high wind speed. To control for any potential effects of tubocurarine chloride on the frogs' ability to adjust their R_c, physically restricted animals were used instead. Frogs were restricted in a meshed cage, and their rates of total EWL, including water loss via the skin and pulmonary tract, were measured at a wind speed of 0.6 cm/s. After rehydration and re-acclimation at 21.0 ± 1.0°C for 1 d, rates of total EWL were measured under the same conditions and in the same individuals except that the wind speed was 1.0 ± 0.1 m/s.

Measurement of the rate of water loss

Rates of CWL and EWL were measured gravimetrically in a horizontal cylindrical wind tunnel (8.5 cm in diameter and 17.0 cm long). The design using these measurements was similar to that described by Wygoda (1984). One end of the tunnel was fitted with a fan to create a wind speed of 1.0 ± 0.1 m/s (measured by an anemometer at the opposite end) or an air pump coupled with a mass flow meter (Riteflow, New York, NY, USA) to distribute 1960 ml/min of room air to produce an approximate wind speed of 0.6 cm/s. Parallel straws (1 cm long × 0.7 cm in diameter) were placed approximately 1 cm from the fan to decrease airflow turbulence (Shoemaker et al. 1987). The air flow was programmed to stop for 10 s at 5-min intervals so that the body mass could be measured. In the middle of the wind tunnel, a 4 × 4-cm glass platform was connected to an electronic balance (Mettler J1000) by a rod that passed through the wind tunnel. All measurements were performed in a room at 21.0 ± 1.0°C and 75.0% ± 5.0% RH.

In each trial, only a paralyzed or restricted frog that was hydrated and had an empty digestive tract was used. The frog's bladder was emptied by gently applying pressure to its abdomen. The change in body mass during the experiment due to metabolism and gas exchange was regarded as negligible. The frog was carefully wiped down with a paper towel and placed on the glass platform. For the paralyzed treatment, the frog was placed in a water-conserving posture, with the limbs tucked under its body and its chin resting against the glass platform (Heatwole et al. 1969). The frog, either paralyzed or restricted, was covered with a 5-mm-mesh cage.

The frog was weighed to the nearest 1 mg by the electronic balance prior to the trial. This body mass was defined as the standard mass (Ruibal 1962). The body mass of the frog was recorded automatically every 5 min by a computer connected to the balance when the air flow was stopped. A preliminary test on 4 individuals (3 arboreal and 1 non-arboreal frogs) showed that the rate of water loss dropped slightly after the 1st 30 min and then remained relatively constant during the next 7 h. Thus, in this study, each trial lasted 4 h, but data from the 1st hour was excluded from the analysis. The dorsal skin temperature of the frog was measured to the nearest 0.1°C by a thermocouple thermometer (TES1300, TES Electrical Electronic, Taipei, Taiwan) within 15 s after the end of the trial. Individuals with excretions found during the experiments were excluded from the analysis.

The area-specific rate of water loss (EWL; $g/cm^2/h$) was calculated for each frog by the equation:

$$\mathrm{EWL} = (W_1 - W_2)/ [SA_E \times (T_2 - T_1)];$$

where W_1, W_2, T_1, and T_2 respectively indicate the initial body mass (standard mass; g), final body mass, start time, and end time of each trial. SA_E (cm^2), the air-exposed surface area, was estimated from the standard mass using an equation described by Withers et al. (1982). This equation was modified from an equation of McClanahan and Baldwin (1969) for frogs in the water-conservation posture. This method was verified for the arboreal hylids *Litoria caerulea* and *L. chloris* (Buttemer 1990) and has been used in numerous studies (Young et al. 2005).

Measurement of the boundary layer and cutaneous resistances

For each live animal, the total resistance R_t ($R_t = R_c + R_b$) was calculated as follows:

$$R_t = [sds(T_s) - RH \times sda(T_a)] / \mathrm{EWL};$$

where $sds(T_s)$ and $sda(T_a)$ refer to the saturation vapor density (g/cm^3) at skin temperature (T_s) and air temperature (T_a), respectively.

R_b of the animal was calculated using a same-sized 3% agar replica. A paralyzed frog or a restricted frog that was anesthetized with 5% tricaine methane sulfonate (Sigma MS-222) was placed in the water-conserving posture. A mold of the frog was made by embedding the animal in dental impression material (Jeltrate). An exact replica of the frog was then made by filling 3% agar into the mold. Because R_c of the agar replica can be regarded as 0 (Spotila and Berman 1976), R_b (also R_t of the agar replica) was calculated by the equation:

$$R_b = [sds(T_{s\text{-}agar}) - RH \times sda(T_a)]/ EWL_{agar};$$

where $sds(T_{s\text{-}agar})$ is the saturation vapor density at the skin temperature of the agar replica ($T_{s\text{-}agar}$). R_c (h/cm) of the live animal was the difference in the animal's R_t and R_b. For comparison to the values of other species in the literature, the units of rates of CWL and EWL were also adjusted to mg/cm^2/h, and the units of R_c and R_b were adjusted to s/cm.

Statistical analysis

All data are presented as the mean ± 1 standard error (SE), and *n* indicates the number of individuals. Student's *t*-test was used to test for differences in the CWL and total resistance (R_t) between animals and the same-sized agar replicas, and between the CWL of paralyzed frogs and the EWL of restricted frogs. We used a one-way analysis of variance (ANOVA) to test for differences of mean R_c values among species. Pair-wise comparisons were determined using the Student-Newman-Kuel post-hoc test. A paired *t*-test was used to compare the rates of EWL and R_c at 2 different wind speeds. A significant difference was set at $p = 0.05$.

RESULTS

For paralyzed frogs, area-specific rates of CWL were significantly lower and R_t values were significantly higher in live animals than in the same-sized agar replicas for all rhacophorid species (all $p < 0.001$), but no significant difference was found for *D. melanostictus* or *R. longicrus* (Table 1). Rates of CWL of arboreal-species were approximately 1/2-1/5 those of non-arboreal species. Accordingly, R_C values of arboreal species (1.2-3.3 s/cm) were significantly higher than those of non-arboreal species (0.1-0.2 s/cm, $p < 0.001$, Table 1).

For restricted frogs, all arboreal species except *K. eiffingeri* became quiescent a few minutes after the experiments began and maintained a water-conserving posture throughout the experiments. Some individuals of *K. eiffingeri* and the terrestrial rhacophorid *B. japonica* struggled during the middle and late stages of the high-wind-speed experiments. Individuals of *D. melanosticus* and *R. longicrus* vigorously struggled and attempted to escape throughout the experiments. At the high wind speed, there were no significant differences between the CWL

Table 1. Lifestyle, body mass, area-specific cutaneous evaporative water loss (CWL), total resistance (R_t), and cutaneous resistance (R_c) of live animals and agar replicas of 10 Taiwanese amphibians at 21 ± 1°C, a wind speed of 1.0 ± 0.1 m/s, and relative humidity of 75% ± 5%

Species	Lifestyle	*n*	Mass (g)	Animal CWL (mg/cm^2/h)	Agar CWL (mg/cm^2/h)	Animal R_t (s/cm)	Agar R_t (s/cm)	R_c (s/cm)
Rhacophoridae								
Rhacophorus taipeianus	Arboreal	6	3.8 ± 0.2	4.7 ± 0.2	16.4 ± 0.6^{+++}	1.8 ± 0.1	0.2 ± 0.01***	1.5 ± 0.2^{c}
Rhacophorus moltrechti	Arboreal	8	6.0 ± 0.4	3.9 ± 0.2	16.0 ± 0.4^{+++}	2.7 ± 0.2	0.2 ± 0.01***	2.5 ± 0.3^{b}
Rhacophorus arvalis	Arboreal	2	5.1 ± 0.6	4.7 ± 0.1	15.2 ± 1.1^{+++}	3.3 ± 0.1	0.3 ± 0.05***	3.0 ± 0.04^{a,b}
Rhacophorus prasinatus	Arboreal	3	11.5 ± 2.5	3.1 ± 0.1	14.7 ± 0.4^{+++}	2.3 ± 0.1	0.3 ± 0.02***	2.0 ± 0.04^{b,c}
Polypedates megacephalus	Arboreal	8	8.6 ± 0.3	2.8 ± 0.2	11.9 ± 0.5^{+++}	3.0 ± 0.2	0.6 ± 0.03***	2.5 ± 0.2^{b}
Kurixalus eiffingeri	Arboreal	9	3.3 ± 0.3	2.8 ± 0.2	12.8 ± 0.6^{+++}	4.1 ± 0.3	0.7 ± 0.04***	3.3 ± 0.4^{a}
Buergeria robusta	Arboreal	8	11.7 ± 0.8	5.6 ± 0.4	14.2 ± 0.7^{+++}	1.6 ± 0.1	0.4 ± 0.05***	1.2 ± 0.1^{c}
Buergeria japonica	Terrestrial	6	1.7 ± 0.1	10.8 ± 0.6	14.5 ± 0.2^{+++}	0.5 ± 0.03	0.4 ± 0.01***	0.2 ± 0.04^{d}
Bufonidae								
Duttaphrynus melanostictus	Terrestrial	8	12.9 ± 1.8	9.8 ± 0.4	9.6 ± 0.4	0.9 ± 0.1	0.7 ± 0.1	0.2 ± 0.1^{d}
Ranidae								
Rana longicrus	Terrestrial	6	10.2 ± 0.9	13.1 ± 0.8	14.2 ± 0.5	0.6 ± 0.1	0.6 ± 0.03	0.1 ± 0.1^{d}

Data are presented as the mean + 1 S.E., and *n* indicates the number of individuals. $^{+++}$ and *** indicate $p < 0.001$ for CWL and R_t, respectively, between animals and same-sized agar replicas. Different letters denote groups with statistically significant differences in R_c.

of paralyzed frogs and the EWL of restricted frogs in any species except *K. eiffingeri* and *B. japonica* (Fig. 1). Both *K. eiffingeri* and *B. japonica* showed significantly higher rates of water loss in restricted frogs than in paralyzed frogs (all $p < 0.03$).

Table 2 shows R_c and R_b values of 3 arboreal species (*Rha. taipeianus*, *Rha. moltrechti*, and *P. megacephalus*) at low and high wind speeds. Although all 10 species were measured, we only present data for those 3 species because they did not move around during the water loss trials, they showed no significant differences between the CWL of paralyzed frogs and the EWL of restricted frogs, and they each had a relatively large sample size ($n = 8$). The results showed that R_b values of all 3 species were significantly lower at 1.0 m/s wind speed than at 0.6 cm/s. Rc, however, did not significantly differ between the 2 wind speeds in any of the 3 species.

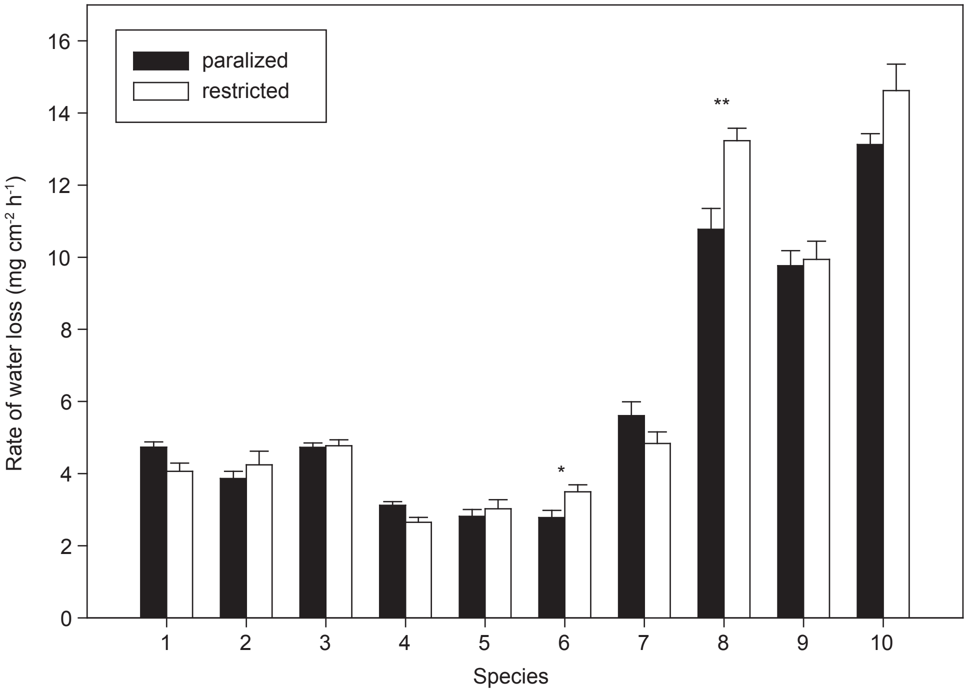

Fig. 1. Area-specific rates of cutaneous evaporative water loss (mean + 1 S.E.) of paralyzed frogs (black bars) and rates of total evaporative water loss of restricted frogs (white bars) of 10 Taiwanese amphibian species at 21 ± 0.1°C and a wind speed of 1.0 ± 0.1 m/s. Species are (1) *Rhacophorus taipeianus*, (2) *Rha. moltrechti*, (3) *Rha. arvalis*, (4) *Rha. prasinatus*, (5) *Polypedates megacephalus*, (6) *Kurixalus eiffingeri*, (7) *Buergeria robusta* (1-7 are arboreal), (8) *B. japonica*, (9) *Duttaphrynus melanostictus*, and (10) *Rana longicrus* (8-10 are terrestrial). * $p < 0.05$; ** $p < 0.01$, for the rate of water loss between 2 experimental groups of the same species.

Table 2. Area-specific rate of evaporative water loss (EWL), boundary layer resistance (R_b), and cutaneous resistances (R_c) of 3 Taiwanese arboreal rhacophorid amphibians at 2 wind speeds

		Low wind speed (0.6 cm/s)			High wind speed (1.0 ± 0.1 m/s)		
Species	Body mass (g)	EWL (mg/cm^2/h)	R_b (s/cm)	R_c (s/cm)	EWL (mg/cm^2/h)	R_b (s/cm)	R_c (s/cm)
Rhacophorus taipeianus	4.9 ± 0.4	1.7 ± 0.1	3.8 ± 0.1	2.0 ± 0.5	4.1 ± 0.2	0.5 ± 0.01	1.8 ± 0.3
Rhacophorus moltrechti	6.9 ± 1.2	2.0 ± 0.2	3.2 ± 0.2	2.8 ± 0.4	4.2 ± 0.4	0.5 ± 0.01	2.5 ± 0.3
Polypedates megacephalus	8.7 ± 0.5	2.1 ± 0.1	2.5 ± 0.1	2.7 ± 0.3	3.0 ± 0.3	0.6 ± 0.03	2.7 ± 0.4

Data are presented as the mean + 1 SE. $n = 8$ for all 3 species.

DISCUSSION

Our results showed that arboreal rhacophorid amphibians in humid subtropical Taiwan exhibit R_c values higher than those of "typical" frogs. This current study adds Taiwanese rhacophorids to a growing list of anuran species that demonstrate a significant R_c. Similar to hylid species, our results showed that R_c values in rhacophorid species are highly related to arboreality. Unexpectedly, we did not find that arboreal rhacophorids increased their R_c when they experienced increased rates of EWL associated with a high wind speed.

Resistance to cutaneous water loss

Although inhabiting moist habitats, Taiwanese arboreal rhacophorids demonstrate detectable R_c values ranging 1.2-3.3 s/cm. These values are similar to many hylids inhabiting mesic regions (Withers et al. 1984, Wygoda 1984, Amey and Grigg 1995, Withers 1998). Consequently, rates of CWL of Taiwanese arboreal rhacophorids were approximately 1/2-1/5 of non-arboreal species, which have low or negligible R_c values. Considering the lower availability of free water and stronger air movements of arboreal habitats compared to ground environments (Yorio and Bentley 1977, Shoemaker et al. 1987), R_c values of these arboreal amphibians could benefit them by significantly reducing their risk of desiccation (Tracy et al. 2010).

Additionally, Tracy and Christian (2005) showed that the R_c of an amphibian species is correlated with its preferred temperature: species with higher R_c select higher temperatures. Thus, the R_c of arboreal frogs might present a thermal opportunity to exploit a wider range of habitat than non-arboreal frogs.

For hylid species, the R_c of a species was shown to be highly related to its arboreality (Wygoda 1984, Young et al. 2005). The same pattern was found in the Rhacophoridae: our results showed that all arboreal rhacophorid species exhibited R_c values of > 1.2 s/cm, whereas the non-arboreal *B. japonica* displayed a low R_c of 0.2 s/cm. It is noteworthy that despite an arboreal lifestyle, the Fiji tree frog *Platymantis vitiensis* (Ranidae) and common couqí *Elutherodactylus coqui* (Leptodactylidae) showed low or negligible R_c values (Rogowitz et al. 1999, Young et al. 2006). This suggests that arboreality is not the sole factor determining the evolution of R_c in amphibians.

There is wide variability in R_c values among atypical anuran amphibians. It was suggested that the value of R_c is correlated with habitat aridity (Shoemaker et al. 1992). The available data on rhacophorids show a similar pattern. For example, the xeric arboreal *Chiromantis petersi* exhibits a high R_c of > 300 s/cm (Withers et al. 1984), whereas rhacophorid species found in moist habitats have a relatively small R_c of < 5 s/cm (this study). However, the high R_c value (404 s/cm) of the rainforest-inhabiting *C. rufescens* (Withers et al. 1984) and the relatively low R_c value (1.9 s/cm) of *P. maculates* that inhabits seasonal hot and arid habitats (Lillywhite et al. 1997) do not appear to support the relationship between the R_c value and habitat aridity. Young et al. (2005) found that closely related hylid species have similar R_c values, suggesting that their phylogenetic histories might play an important role in determining the R_c value of a species. Taken together, the R_c value of a species could be a consequence of a combination of many factors and cannot be explained solely by the phylogenetic history or environmental factors such as aridity of the habitat.

We found a low but statistically significant R_c in the non-arboreal rhacophorid *B. japonica*. Several non-arboreal species were reported to exhibit a low but significant R_c, such as *Elutherodactylus cooki* (Rogowitz et al. 1999), *Litoria* (Young et al. 2005), *Rhinella* (Prates and Navas 1999), and *Fejervarya cancrivora* (Wygoda et al. 2011). Wygoda et al. (2011) showed that a low R_c value of 0.27 s/cm allowed a 2.4-g crab-eating frog *F. cancrivora* to stay out of water for 1.5 h longer than a same-sized frog with no R_c. The relatively low R_c in non-arboreal species can benefit animals to some degree in terms of reducing EWL and thus should not be considered negligible.

Most studies on EWL in amphibians were conducted under carefully controlled conditions in the laboratory. Future work might focus on measuring EWL and R_b of free-ranging frogs and on testing whether a higher R_c value allows a species to stray further and stay longer from water sources.

Effects of wind speed on EWL and R_c

Our results showed that a wind speed of approximately 1.0 m/s destroyed most of the R_b, as shown in previous studies (Foley and Spotila 1978). However, despite the significantly higher rates of EWL at the high wind speed, Taiwanese arboreal rhacophorids did not adjust their R_c. This suggests that the R_c of Taiwanese rhacophorids might not be as labile as expected in response to environmental demands. Wygoda (1988) suggested that the increased R_c of *Hyla cinerea* at a decreased RH was because this species increased the frequency and intensity of mucus discharge of the skin, and the increased layers of dried mucus served as a water barrier. This might not be the case for Taiwanese rhacophorids. We observed no visible secretions being expelled from glands on the dorsal skin during the trials.

Water loss from the pulmonary tract

In the present study, the lack of a significant difference between the EWL of restricted frogs and the CWL of paralyzed frogs in 6 arboreal species indicates that pulmonary water loss in Taiwanese arboreal rhacophorids accounts for only a small portion of the total EWL and thus can be disregarded. Previous studies showed that pulmonary water loss accounted for < 1% of the total EWL in some typical species such as *Scaphiopus holbrooki* (Wygoda 1981), *Lithobates pipiens* (Spotila and Berman 1976, Bentley and Yorio 1979), and *Rhinella marinus* (Bentley and Yorio 1979), and in an arboreal hylid, *Agalychnis dacnicolor* (Bentley and Yorio 1979). However, with an increased R_c, some waterproof amphibians were shown to substantially increase the proportions of gases exchanged via the pulmonary tract compared to typical anurans (Giese and Linsenmair 1986, Stinner and Shoemaker 1987). Water loss from the pulmonary tract was shown to account for approximately 15% of the total EWL in the waterproof frog *Hyperolius viridiflavus nitidulus* during the dry season (Giese and Linsenmair 1986). Despite a slight R_c, our results showed that respiratory gas exchange, and thus the EWL, of Taiwanese rhacophorids occurs mainly through the skin.

For *B. japonica* and *K. eiffingeri*, rates of water loss of restricted frogs were 25% higher than those of paralyzed frogs. Restricted frogs of both species engaged in some movements during the trials. Movements, which increase a frog's exposed surface area and disturb its boundary layer, were shown to increase water loss by up to 200% in amphibians (Heatwole et al. 1969). The increased rate of water loss in restricted frogs of *B. japonica* and *K. eiffingeri* was likely due to movement rather than to respiration through the pulmonary tract.

Evaluation of cutaneous resistance mechanisms

Mechanisms for R_c in non-cocooned anuran amphibians are better understood for *Phyllomedusa*, which secretes lipid materials by specialized lipid glands and spreads the waxy secretions over its body with a wiping behavior (Blaylock et al. 1976). Other suggested waterproofing mechanisms include a specialized cutaneous lipid layer in hylid frogs *Litoria fallax* and *L. peroni* (Amey and Grigg 1995), multiple layers of densely packed purine-rich iridophores that can reflect light and heat or retard water diffusion in *Chiromantis* and *Hyperolius* (Drewes et al. 1977, Kobelt and Linsenmair 1986 1992), and layers of dried mucus that cover the skin glands (Geise and Linsenmair 1986, Wygoda 1988). Studies also suggested that a mixture of lipids and proteins in skin secretions coupled, in some cases, with wiping behaviors might be related to the reduced CWL in some amphibians (McClanahan et al. 1978, Christian and Parry 1997, Lillywhite et al. 1997, Barbeau and Lillywhite 2005). In the present study, no wiping behaviors were observed in any species during the trials or acclimation periods. Additionally, Taiwanese rhacophorid frogs demonstrated a typical anuran skin without specialized lipid glands, lipid layers, or multiple layers of iridophores on their dorsal skin (Liu 1996). The mechanism underlying differences in R_c values of Taiwanese rhacophorids requires further investigation.

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