Nepenthes pitchers *versus* thermogenic flowers: thermal patterns and their role in prey capture and pollination

Gokul Baburaj Sujatha*[,±], Anil John Johnson*, Abdul Azeez Hussain[¥], Sabulal Baby*[,1]

***Phytochemistry and Phytopharmacology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pacha-Palode, Thiruvananthapuram 695562, Kerala, India.

[±]University of Kerala, Thiruvananthapuram 695034, Kerala, India.

[¥]Garden Management Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pacha-Palode, Thiruvananthapuram 695562, Kerala, India

[1]E-mail: sabulal@gmail.com, sabulal@jntbgri.res.in

Abstract

Prey capture in *Nepenthes* and pollination in angiosperms are two antithetical events; one designed to trap insects and other arthropods (carnivory) and the other to transfer pollen through pollinators (reproduction). In this study, infrared thermography was extensively used to obtain thermal profiles of *Nepenthes* pitchers and thermogenic and non-thermogenic flowers in field conditions. Confocal and scanning electron microscopy were used to record peristome cross-sectional features and pitcher morphology. *N. khasiana* pitchers displayed below ambient temperatures during evening-night-morning hours (5 pm to 8-9-10 am); pitcher spots recorded lowest and highest temperatures as 14.4 (6-7 am) and 45.6^{o}C (2 pm), respectively. The average humidities in the night and day periods were 77.15% and 50.15%, respectively. The prey capturing zones in *Nepenthes* pitchers (peristome, lid and their intersection) are colder favoring prey capture, whereas in thermogenic flowers, floral portions are hotter, providing the thermal requirements for the pollinator. In thermogenic plants, floral zones assist pollination by offering a 'thermal reward' through enzymatic processes; but *Nepenthes* traps achieve lower temperature spots by physical (surface microstructures), chemical (nectar) and ecological (rain, humidity) factors. Both are contrasting 'life' and 'death' adaptations in nature.

Introduction

Nepenthes pitchers are leaf-evolved biological traps; they entice-trap insects and other arthropods, involve in mutualistic interactions with small mammals and eventually gain nutrients leading to their survival in low supplement growing environments. Carnivory of these pitcher traps is concocted through various attraction, deception, capture and digestion strategies (Raj et al. 2011; Kurup et al. 2013; Baby et al. 2017). *Nepenthes* pitchers consist of distinct zones based on their function, (i) prey capture zone (peristome/lid), (ii) slippery zone (interior waxy region) and (iii) digestive zone (interior bottom portion of digestive fluid) (Bauer and Federle 2009; Wang and Zhou 2016; Baby et al. 2017). The top zone (peristome, lid) of *Nepenthes* pitchers is crucial in attraction and capture of insects and other small organisms. We found UV induced fluorescence emissions primarily from the peristomes of various *Nepenthes* species/hybrids (Kurup et al. 2013). Similar fluorescence emissions from reproductive structures (anther, pollen) were proved to be visual cues attracting pollinators to flowers (Baby et al. 2013; Mori et al. 2018). Fluorescence also functions as signals in various amphibians (frogs) in terrestrial environments, marine organisms, segmented worms, reptiles and other organisms (Taboada et al*.* 2017; Mori et al*.* 2018; Kohler et al*.* 2019; Ben-Zvi et al*.* 2022). In *Nepenthes*, the peristome is a wettable surface with distinct microstructures (Bohn and Federle, 2004; Chen et al. 2016). There are nectaries towards the inner sides of most *Nepenthes* pitcher peristomes. The peristome wettability is through two major factors, nectar and rain water (Bohn and Federle 2004; Chen et al. 2016); and the wet peristome surface is slippery for the visiting preys. Bauer et al. 2008 reported high rates of prey capture in the evening, night and early morning in *Nepenthes* pitchers. In pollination, a more common natural phenomenon, insects are attracted to flowers through various strategies, and among others (pollen, nectar), thermal reward is one of the factors engaging insects to the flowers in several plants (Seymour 1997). Contrastingly, in flowers after their visit (pollination) insects ‘fly away’ whereas in *Nepenthes* pitchers the visitors (preys) are destined to ‘fall’ into these traps.

This study is primarily designed to reflect on the influence of thermal patterns in prey capture (pitchers) in *Nepenthes* and pollination (flowers) in angiosperms. Infrared

radiation is emitted by any object at temperatures above absolute zero, and infrared thermography is a technique which detects infrared energy emitted from the object, converts it to temperature, and displays the image of temperature distribution. It is a non-invasive technique and requires no contact to measure the temperature accurately (Merlot et al. 2002; Wang et al. 2004; Dyer et al. 2006; Usamentiaga et al. 2014; Tattersall et al. 2016; Harrap et al. 2017). Here we report (i) the thermal profiles of *Nepenthes* pitchers in field and lab conditions, (ii) their comparison with thermal patterns of thermogenic and non-thermogenic flowers and (iii) discuss the influence of thermal patterns in prey capture (carnivory) and pollination (reproduction).

Materials and methods

Study species

Pitchers of *Nepenthes khasiana* Hook. f. (Fig. 1) and *Nepenthes* hybrids (Fig. S1) were sampled from the garden sites of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) (08°45′00.04″N, 77°01′41.09″E). Flowers of *Victoria amazonica* (Poepp.) Klotzsch (family: Nymphaeaceae, common name: giant water lily or Amazon water lily), *Nelumbo nucifera* Gaertn. (Nelumbonaceae, sacred or Indian lotus), *Spathiphyllum cannifolium* (Dryand. ex Sims) Schott, *S. wallisii* Regel (Araceae), *Aglaonema commutatum* Schott (Araceae), and rose hybrids (Rosaceae) (Fig. 2) were thermographed from the garden sites.

Infrared thermography

Nepenthes khasiana, *Nepenthes* hybrid pitchers, thermogenic and non-thermogenic flowers were subjected to infrared thermography in the field and their thermal profiles were recorded using infrared camera with FOL lens (18 mm) and IR resolution (240 x 320) (FLIR 420bx, USA). The thermal patterns were analyzed using FLIR Tools Version 5.5. Infrared thermographic profiles of pitchers and flowers were recorded in the field in short time intervals and in 1 hour intervals for 48 hours (day & night). *N. khasiana* pitchers were also thermographed in the lab under controlled conditions. Temperatures of 3 spots (sp1, sp2, sp3) in *N. khasiana* unopen pitchers (P1U-P6U), 5 spots (sp1, sp2, sp3, sp4, sp5) in open pitchers (P1O-P6O) and chosen spots on thermogenic/non-thermogenic

flowers were recorded from their thermal images (Figs. 1, 2, S4, S5, S6). Infrared thermographic measurements of unopen/open *N. khasiana* pitchers (P1U-P3U & P1O-P3O) were recorded in January 2019 and (P4U-P6U & P4O-P6O) in March 2021. All thermographic measurements in the field were carried out on non-rainy days. An Environmental Meter (Extech Instruments 45170, FLIR Systems, India) was used to measure the relative humidity and atmospheric temperature.

Confocal microscopy

Small sections of *N. khasiana* peristomes were directly imaged using Confocal Laser Scanning Microscope (CLSM, Leica SP8 WLL, Germany) and 3D stack images were obtained using 20x objective, with excitation/emission 405/425-475.

Scanning electron microscopy

Scanning electron microscopy (SEM) imaging of *N. khasiana* sections was carried out on a SEM (Hitachi S-2400, Japan). *N. khasiana* pitcher sections were fixed in 3% glutaraldehyde in phosphate buffer and kept overnight. Sections were then dehydrated sequentially with different concentrations of ethanol (30%, 50%, 70% 15 min each, two changes and 90% and 100% ethanol, 35 min each, two changes). The dehydrated sections were then subjected to critical point drying, gold coated and viewed on the SEM. Again, *N. khasiana* peristome and lid samples were mounted over aluminium stub using double sided carbon tape and imaged using high resolution direct SEM (FEI Quanta 200, Netherlands).

Results

Nepenthes pitchers, thermal patterns

Thermal profiles of 3 spots (sp1, sp2, sp3; spots labelled in Fig. 1) in unopen *N. khasiana* pitchers (P1U to P6U) (Figs. 1, 3) and 5 spots (sp4: peristome-lid joint and sp5: centre of the open lid; spots labelled in Fig. 1) in open pitchers (P1O to P6O) (Fig. 1, 4) were measured in field and laboratory conditions. The 2 top spots (sp4, sp5) are exposed (or measurable) only in open pitchers. *N. khasiana* pitchers showed temperature zones at its various regions (spots). For example, in the unopened pitcher (P2U) (Fig. 3B) from 5 pm

to 8 am (average humidity and temperature: 79.4 ± 4.42% and 23.54 ± 2.68^{o}C), pitcher temperature zones (sp1-sp3) remained below the atm. temperature and from 9 am to 4 pm (average humidity and temperature: 56.01 ± 13.13% and 31.98 ± 3.07^{o}C) pitcher zones exceeded the ambient temperature. Among the pitcher zones, sp3 (blue, pitcher top) attained a maximum temperature of 43.3^{o}C at 11 am, and sp2 (violet) and sp3 (blue) attained the lowest temperatures of 15.7^{o}C at 6 am. Maximum temperature difference between the ambient temperature and pitcher zones (sp1, sp2, sp3) in the nighttime (5 pm to 8 am) was (24.7-18.9^{o}C (sp3)) 5.8^{o}C at 10 pm. Similar temperature difference in daytime (9 am to 4 pm) was (43.3 (sp3) - 30.7^{o}C) a significant 12.6^{o}C (above ambient). In the nighttime, all three pitcher spots (sp1, sp2, sp3) are almost overlapping throughout, but in daytime spots (pitcher regions) are distinct, with maximum temp difference of 43.3 (sp3) - 38.2 (sp1), i.e., 5.1 degrees at 11 am. The top spot (sp3) of *Nepenthes* unopened pitchers were the hottest.

In an example of an open *N. khasiana* pitcher (P1O) (Fig. 4A), the nighttime zone extended from 5 pm to 8 am (morning) next day. In this time range, the 5 pitcher spots (sp1-sp5) showed temperatures below the atmospheric temperatures. Ambient temp. range (5 pm to 8 am) was from 20.4-29.7 degrees with an average 23.54 ± 2.68 degrees and humidity ranged from 67.5-83.5% (average, 79.40 ± 4.42%). Lowest pitcher temp. (spot) in the night zone was 16.7 degrees (6 am, sp5) and highest temp. was 29.1 degrees (5 pm, sp1). In the daytime zone (9 am to 4 pm), most pitcher spots (zones) exceeded the ambient temperature. Ambient temp. and humidity ranges were 26.3-35.4 degrees (average, 31.98 ± 3.07 degrees) and 41.5-79.0% (56.01 ± 13.13%), respectively. Lowest temp in the day zone was 23.2 degrees (9 am, sp4) and highest was 38.2 (1 pm, sp5).

In open (P1O to P6O) and unopen (P1U to P6U) pitchers (6 each) monitored for 48 h in the field (Figs. 3, 4), the pitcher temperatures were lower than the atmospheric temperatures starting evening (5 pm), throughout the night (6 pm to 6 am), and in the morning hours (8 am or 9 am or 10 am). These lower temperatures of the pitcher zones extended to daytime (up to 10 am), depending on the type of the pitchers (open/unopen) and their ecological conditions. In six each unopen and open pitchers, spots (sp1-sp3 &

sp1-sp5) remained > 3 degrees below the atmospheric temperatures in the evening-night-morning hours (Tables S1-S12, Figs. S2, S3); and the pitcher spots (unopen pitchers: sp1-sp3; open pitchers: sp1-sp5) were mostly overlapping. The atmospheric-spot temperature difference in the nigh time was 6.7^{o}C (each) in the unopen pitcher P4U (sp3, 4 am) and open pitcher P6O (sp5, 9 pm).

The lowest temperatures in six unopened (P1U-P6U) pitchers were 17.3 (P1U: 4 am, sp3), 15.7 (P2U: 6 am, sp2/sp3), 16.2 (P3U: 6 am, sp3), 14.4 (P4U: 7 am, sp3), 14.4 (P5U: 6 am, sp3), 14.8 (P6U: 6 am, sp3) degrees and in six opened (P1O-P6O) pitchers were 16.7 (P1O: 6 am, sp5), 16.1 (P2O: 5-6 am, sp5), 16.5 (P3O: 6 am, sp3), 15.2 (P4O: 6 am, sp3), 15.0 (P5O: sp2-sp5, 6 am), 14.6 (P6O: 6 am, sp5), respectively. So, in the measurement conditions, *N. khasiana* pitchers recorded lowest temperatures in the range 14.4-17.3 degrees at 4-7 am. In all six unopened pitchers (P1U-P6U) sp3 showed the lowest temperatures (14.4-17.3 degrees) at the morning hours. In opened pitchers (P1O-P6O), sp3 & sp5 recorded the lowest temperatures (14.6-16.7 degrees) under the measurement conditions.

Similarly, the highest temperatures in unopened pitchers (P1U-P6U) were 35.3 (P1U: 11 am, sp1), 43.3 (P2U: 11 am, sp3), 44.3 (P3U: 3 pm, sp3), 45.4 (P4U: 2 pm, sp3), 45.6 (P5U: 2 pm, sp3), 41.3 (P6U: 10 am, sp3). Open pitchers (P1O-P6O) recorded highest temperatures as: 38.2 (P1O: 1 pm, sp5), 40.3 (P2O: 2 pm, sp5), 34.2 (P3O: 2 pm, sp5), 44.3 (P4O: 11 am, sp5), 41.9 (P5O: 11 am, sp5 & 3 pm, sp2), 42.0 (P6O: 2 pm, sp5). The highest pitcher temperatures (34.2-45.6 degrees) were registered from 10 am-3 pm. In 6 unopened pitchers (P1U-P6U), sp3 (5) & sp1 (1) recorded highest temperatures (35.3-45.6 degrees). In 6 open pitchers (P1O-P6O), sp5 (6) & sp2 (1) recorded highest temperatures (34.2-44.3 degrees).

In unopen/open pitchers (P1O-P6O & P1U-P6U) in 24 h (under the field experimental conditions), the lowest and highest temperatures between pitcher spots varied markedly, unopen pitchers: (P1U: 35.3 (sp1)-17.3 (sp3) = 18°C; P2U: 43.3 (sp3)-15.7(sp2/sp3) = 27.6^{o}C; P3U: 44.3 (sp3) - 16.2 (sp3) = 28.1^{o}C; P4U: 45.4 (sp3)-14.4 (sp3) = 31°C; P5U:

45.6 (sp3)-14.4 (sp3) = 31.2^{o}C; P6U: 41.3 (sp3)-14.8(sp3) = 26.5^{o}C); open pitchers: (P1O: 38.2 (sp5)-16.7 (sp5) = 21.5^{o}C; P2O: 40.3 (sp5)-16.1 (sp5) = 24.2^{o}C; P3O: 34.2 (sp5)-16.5 (sp3) = 17.7^{o}C; P4O: 44.3 (sp5)-15.2 (sp3) = 29.1^{o}C; P5O: 41.9 (sp5)-15.0(sp2-sp5) = 26.9^{o}C; P6O: 42.0 (sp5)-14.6 (sp5) = 27.4^{o}C). Unopen and open pitchers (6 each) in 24 hours in field measurements showed maximum variations of 31.2 and 29.1^{o}C, respectively, between their spots.

In unopen pitchers (P1U-P6U), the temperature variations (lowest/highest) of pitcher spots against atmospheric temperature (of the respective spot/time): (P1U: low, 4.4^{o}C, 10 pm, 2 am, 4 am (sp3), high, 4.6^{o}C, 11 am (sp1); P2U: low, 5.8^{o}C, 10 pm (sp3), high, 12.6^{o}C, 11 am (sp3); P3U: low, 4.8^{o}C, 10 pm (sp3), high, 8.9^{o}C, 1 pm, 3 pm (sp3); P4U: low, 6.7^{o}C, 4 am (sp3), high, 9.9^{o}C, 2 pm (sp3); P5U: low, 6.5^{o}C, 9 pm (sp3), high, 10.1^{o}C, 2 pm (sp3); P6U: low, 6.3^{o}C, 12 am (sp3), high, 10.6^{o}C, 10 am (sp3)). Similar variations in open pitchers (P1O-P6O): (P1O: low, 4.8^{o}C, 2 am (sp5), high, 6.5^{o}C, 11 am (sp2); P2O: low, 5.4^{o}C, 9 pm, (sp5); high, 5.5^{o}C, 4 pm (sp5); P3O: low, 4.4^{o}C, 9 pm (sp5), high, 1.5^{o}C, 11 am (sp5); P4O: low, 5.5^{o}C, 7 am (sp2, sp3, sp4, sp5), high 11.8^{o}C, 11 am (sp5); P5O: low, 5.8^{o}C, 5 am (sp4), high, 9.4^{o}C, 11 am (sp5); P6O: low, 6.7^{o}C, 9 pm (sp5), high, 6.5^{o}C, 2 pm (sp5). *Nepenthes* hybrid pitchers (open and unopen) also showed similar thermographic patterns (Fig. S1).

In January 2019 measurements (P1U to P3U & P1O to P3O), average humidity and ambient temperature from 5 pm to 9 am were 79.38% and 23.71 degrees, respectively (Fig. S2, S3). Average humidity and ambient temperature from 10 am to 4 pm were 52.73% and 32.79 degrees. Similarly, in March 2021 (P4U to P6U & P4O to P6O), average humidity and ambient temperature from 5 pm to 9 am were 74.91% and 24.54 degrees whereas similar parameters from 10 am to 4 pm were 47.56% and 33.74 degrees, respectively (Fig. S2, S3).

In our infrared thermographic observations, we monitored the opening of one *N. khasiana* pitcher (Fig. 5) in the field. The initiation of pitcher (lid) opening was in the early morning hours (4.30 am) (Fig. 5B), and at this point the pitcher and linked tendril/lamina were wet

throughout, displaying a blue colour in infrared thermography (with maximum colour intensity at the peristome/lid interface). The lid opening extended to the day zone (10.30 am), and the blue (wet) ring was retained at the peristome-lid interphase till 10.30 am (Fig. 5H). Most of the pitcher surface (and the tendril/lamina linked to the pitcher) became hotter (gradually turned yellow) by 8.30 am (Fig. 5F), and at 11.30 am the entire pitcher became almost uniformly hot (yellow) (Fig. 5I). This pattern clearly indicates the moisture-retaining capacity of the peristome-lid interphase (Fig. 5F-H) in *Nepenthes* pitchers.

The variation in pitcher temperatures over time (of the day) and between the pitcher spots is largely influenced by the ecological parameters (ambient temp., humidity, wind, rain etc.). To clarify this, we measured the thermal patterns of *N. khasiana* pitchers in a controlled atmosphere, at stable temperature of (22°C) and humidity (60%). A *N. khasiana* potted plant with multiple pitchers was brought to a laboratory room with controlled atmosphere (22°C/60%), allowed it to stabilize within the room and then the temperature patterns at five spots (sp1-sp5) in a mature open pitcher were measured. In these conditions, all five spots (sp1 to sp5) exceeded the ambient temp. (22°C), with the lowest temp. 22.4^{o}C (sp4) and highest temp. 24.5 degrees (sp2 & sp5) (Fig. 6). sp3 (average 23.06^{o}C) and sp4 (22.74^{o}C) stayed as the lowest temperature spots throughout the measurements under these controlled conditions. The differences between spots (average temp., sp1 to sp5) and ambient (22°C) temperatures were 2.08^{o}C (sp1), 2.18^{o}C (sp2), 1.06^{o}C (sp3), 0.75^{o}C (sp4) and 2.16^{o}C (sp5) (Fig. 6). On spraying water uniformly, the entire pitcher turned blue (indicating low temperatures). The air flow also had a major role on the surface temperatures of pitchers. These observations point to the influence of environment on temperature patterns of *Nepenthes* pitchers in the field. The maximum temperature variation between the spots in controlled condition (22°C/60%) is only 2.1^{o}C, whereas in field these variations are comparatively high. This proves that the high humidity and low temperatures in the evening-night-morning conditions favor the adherence moisture to the pitcher surface, lowering its temperature; the infrared thermographic pictures are showing uniform pattern (blue colour throughout the pitcher) in the nighttime (Figs. 3, 4, 5). As the atmospheric temperature rises from the morning to

noon time, the moisture evaporates from the pitcher surface and the zones (coloured zones) appear in the pitcher surface (Figs. 3, 4, 5).

Thermogenic flowers, thermal patterns

The flowers of two known thermogenic plants, *V. amazonica* and *N. nucifera*, in the same field were subjected to infrared thermographic analysis. In *V. amazonica* flower (second day of flowering, 4 pm to 10 am, 18 h, 19 readings, January 2019), the floral (outer) base portion (sp2) is warmer than atm. temp., and the central portion of the flower (upper floral lobes, sp3) and the base lobes (sp1) remained mostly below atm. temperatures (Figs. 7A, S4, Table S13). In a second set of measurements (two days of flowering, 4 pm to 3 pm, 48 h, January 2022), *V. amazonica* flower bud was fully visible (above the water level) at 16.00 pm in the evening. At 17.00 pm, the hot spot (ring) appeared and the flower (white) completely bloomed by 19.00 pm; and at this stage, all three zones (sp1, sp2, sp3) were clearly visible. After midnight, the temperature of the hottest floral spot (sp2) was lower than the atmospheric temperature, and at early morning sp2 temperature increased. After 11 am till afternoon the atmospheric temperature was higher than the floral spots and the flower turned pink in colour. Along with colour change in the morning hours the flower began to close partially and opened again in the afternoon. This night and day temperature changes continued in the second day. The outer floral petals began to partially drown in the water, the hot ring faded in the second day night and the central portion of the flower began to appear. In the second day morning, the flower was seen floating over the water, differentiation of the spots was difficult and the observations ended at 15.00 pm. In these measurements (two days of flowering), sp2 was mostly hotter, whereas sp1 and sp3 stayed below the ambient atmospheric temperatures (Figs. 7B, S4, Table S14).

In *N. nucifera* (24 h, March 2021) the interior spot (sp3) was warmer compared to the two outer spots (sp2 and sp1); sp3 is higher than ambient temperatures throughout the 24 h field measurements (Figs. 7C, S5, Table S15). sp1 and sp2 remained below ambient temperatures from 4 pm to 10 am. Even in these time periods (evening-night-morning), sp3 retained higher temperatures compared to sp1, sp2 and ambient temperatures. In

the 24 h measurements, average sp1, sp2 and sp3 temperatures were 24.93ºC, 26.55ºC and 29.36ºC (average humidity 66.93%, ave. atmospheric temp. 27.22ºC). The lowest floral temp. was 15.7ºC (sp1, 6.30 am) and at this same time sp2 and sp3 recorded 16.6 and 20.4ºC (sp3-sp1, 4.7ºC); the highest floral temp. was 42.7ºC (sp2, 1.30 pm) and sp1 and sp3 at 1.30 pm were at 36.9 and 39.2ºC, respectively (sp2-sp1, 5.8ºC).

Control flowers, thermal patterns

We also recorded the floral thermal profiles of *S. cannifolium*, *S. wallisii* (Araceae), *A. commutatum* (Araceae), and two rose hybrids using infrared thermography (Figs. 2, S6, S7, Tables S16-20). In *S. cannifolium*, the 24 h thermal patterns of the three spots (sp1, sp2, sp3) are within the atmospheric temperature (Figs. S6, S7). *A. commutatum* also showed similar thermal profiles (Figs. S6, S7). In *S. wallisii*, spots (sp1, sp2, sp3) emerged out of the ambient temperatures from 11 am till 3 pm, and in rest of the 24 h measurements, these spots were below ambient temperature readings (Figs. S6, S7). In the two rose hybrids, sp1 and sp2 were within the ambient temp patterns (24 h), except both the spots emerged out in mid-day hours in one of the hybrids (Figs. S6, S7).

Prey capture strategies

We reported UV induced fluorescence emissions primarily from the prey captures spots of carnivorous plants (Fig. 8) (Kurup et al. 2013). In confocal microscopy, *N. khasiana* pitcher peristome sections (lateral and cross sectional) vividly displayed the blue fluorescence emissions (Fig. 8); its peristome microstructures (fluorescent in UV) were also visible (Figs. 8, 9). Moreover, *N. khasiana* peristome acetone extract displayed multiple blue fluorescent signals on HPTLC profiling (Fig. 10). SEM images displayed glands at the inner sides of pitcher lids, nectaries at the inner sides of peristome, modified stomata at the inner side (below peristome) and secretory glands at the digestive one of *N. khasiana* pitchers (Fig. 9). Direct SEM displayed nectar deposition and glands on pitcher lids (Fig. 9).

Discussion

Nepenthes pitchers, thermal patterns

In *N. khasiana* open (P1O to P6O) and unopen (P1U to P6U) field pitchers (Figs. 3, 4), starting evening (5 pm), throughout the night and in the morning hours (8 or 9 or 10 am), pitcher temperatures remained below the ambient atmospheric temperatures. The pitcher zones (spots) in six each unopen (sp1-sp3) and open (sp1-sp5) pitchers stayed ≥ 3 degrees below the atmospheric temperatures in the evening-night-morning hours (Figs. 3, 4, S2, S3). This difference (atmospheric temperature-spot) was a maximum of 6.7^{o}C (each) in the unopen pitcher (P4U, sp3 - peristome spot, 4 am) and in open pitcher at 9 pm (P6O, sp5 - lid central spot) (Tables S4, S12). Infrared thermographic profiles demonstrate low temperatures of *Nepenthes* pitcher zones in the evening-night-morning hours (average temperatures, 5 pm to 9 am, unopen pitchers: sp1 20.72, sp2 20.55, sp3 19.95 degrees; open pitchers: sp1 20.96, sp2 20.67, sp3 20.33, sp4 20.24, sp5 19.95 degrees) compared to atmospheric temperatures (average 5 pm to 9 am: 24.13 degrees). In daytime, average atm. temperature was comparatively high, 33.27 degrees (10 am to 4 pm), but pitcher zones were still hotter (average temperatures, 10 am to 4 pm, unopen pitchers: sp1 35.65, sp2 35.50, sp3 36.75 degrees; open pitchers: sp1 34.52, sp2 34.28, sp3 33.47, sp4 33.96, sp5 34.65 degrees). The average humidity in these night and day periods were 77.15% and 50.15%, respectively; on the average 27 units higher in the high evening-night-morning hours (5 pm to 9 am) (Figs. 3, 4; Tables S1-S12). In the high humidity night conditions, the moisture from the atmosphere condenses to the peristome/lid portions of the pitcher. Pitcher peristome may also be wetted by rainfall (Moran and Clarke 2010). Further, the nectar produced by nectaries and secretions of glands render wettability to the multiscale peristome cells (Figs. 8, 9).

Coincidentally, most *Nepenthes* species are found restricted to perhumid (high humidity index), mountainous regions with warm days and cold and humid nights in their major centres of diversity, *viz.*, Borneo, Sumatra and the Philippines (Moran et al. 2013; Clarke and Moran 2016; Gray et al. 2017). Further Moran and co-workers (2013) demonstrated that prey capture mechanism in carnivorous plants is constrained by climate, *viz.*, *Nepenthes* species employing peristome-based and viscoelastic fluid-based capture are largely restricted to perhumid regions, and species with wax-based mechanism allows successful capture in both perhumid and more seasonal areas. It has also been observed

that *Nepenthes* in low light and humidity and high nutrient conditions fails to produce pitchers (Pavlovic et al*.* 2007). These reports and the present thermal profiles underline the significance of high humidity in the functioning of *Nepenthes* traps.

In controlled lab conditions (temperature 22ºC, humidity 60%) (Fig. 6), sp3 (peristome: average temp. 23.1ºC) and sp4 (peristome-lid joint: 22.7ºC) showed low temperatures compared to other pitcher zones (sp1 (24.1ºC), sp2 (24.2ºC), sp5 (24.2ºC). This demonstrates that surface characteristics (Figs. 8, 9A-I) favor wettability in *Nepenthes* peristomes. But the peristome and peristome-lid joint spots (sp3, sp4) stayed above ambient temperatures under these controlled conditions (Fig. 6). This evidences the role of high humidity (> 60%, as in night times; Tables S1-S12) and other ecological parameters in maintaining low temperature and wettability of the peristome surface in field conditions.

Thermographic tracking of the pitcher (lid) opening in *N. khasiana* demonstrates the initial 'wet' pitcher (coloured blue) in night zone (12.30 am, Fig. 5A), which gradually switches to yellow (relatively hotter) in the daytime (Fig. 5F-I); the peristome surface retains the 'wetness' till 10.30 am (Fig. 5H). The pitcher rim (or peristome) in *Nepenthes*, which often extending upwards to the lid forming a distinct neck (Bauer and Federle, 2009), is the hub of prey capture strategies (Figs. 1, 5, 8, 9). The nectar (considered as the major 'offer' or 'reward'; Bauer and Federle 2009; Moran and Clarke 2010) in most *Nepenthes* species is secreted in the extrafloral nectaries located at the inner edge of the peristome (Fig. 9F-I), prompting ants and insects to reach towards the cold (wet) region at the deep interior of the pitcher surface. Bauer and co-workers (2009) illustrated that in wet conditions most ants fell (slipped) into the pitcher when they stepped onto the peristome whereas dry peristome surfaces were not slippery. Simply, peristome wetness and pitcher capture efficiency are in near harmony. Further, experimental peristome wetting in *N. rafflesiana* var. *typica* (Bauer et al*.* 2008) and *N. bicalcarata* (Bohn and Federle, 2004) demonstrated increased capture efficiency to > 60%. In *N. rafflesiana* var. *typica* pitchers capture efficiency was very high in the evening-night-early morning whereas it was low during the daytime (Bauer et al*.* 2008). Again, in the evening hours droplets of liquid nectar were

noticed on the peristomes of *N. rafflesiana* var. *typica*, and this suggests that nectar secretion plays an important role in peristome wetting (Bauer et al. 2008). In direct SEM, *N. khasiana* also displayed nectar deposition in its lids (Fig. 9D, E). Another study showed that the peristome nectaries of *N. rafflesiana* only start secreting after the pitcher has opened, and this coincides with prey capture readiness (Bauer and Federle 2009). Bauer and co-workers further suggested that *Nepenthes* nectar is mainly secreted at times of high humidity when the peristome is already fully wetted, that is, between the evening and early morning (Bauer et al. 2008). Our data of thermal profiles of *Nepenthes* pitchers are further correlating nectar secretion (and peristome wettability) with night time low temperature and high humidity in field conditions.

Chen and co-workers (2016) demonstrated continuous, directional water transport on the surface of *Nepenthes* peristomes. This is due to its multiscale epidermal cells, which optimizes and enhances capillary rise in the transport direction, and prevents backflow by pinning any waterfront that is moving in the reverse direction. This anisotropy (the individual epidermal cells overlap in the same direction, from the outer edge of the peristome inwards) results not only in unidirectional flow (despite the absence of any surface-energy gradient) but also enhances surface wettability (Bauer and Federle 2004; Bauer and Federle 2009; Chen et al. 2016). Similar, peristome multiscale structures were also observed in *N. khasiana* (Fig. 9F-I). These unique super-hydrophilic surface properties of the peristome is a critical factor in prey capture. The combination of a hierarchical surface structure and hygroscopic nectar secretions results in an exceptional water-lubricated trapping system (Fig. 9D-I). The three-dimensional plate-like wax crystals and modified stomata in the hydrophobic slippery zone further prevents the prey from escaping (Fig. 9J, K) (Hsu et al. 2015; Baby et al. 2017). The hygroscopic nectar secreted at the inner margin of the peristome and hydrophilic surface chemistry associated to surface roughness make the peristome completely covered by a homogenous liquid film which makes it extremely slippery for insects (Fig. 9D-K) (Bohn and Federle 2004; Bauer et al. 2008; Bauer and Federle 2009; Miguel et al. 2018). The high wettability of the *Nepenthes* peristome is achieved by a combination of hydrophilicity, surface micro-topography, hygroscopic nectar and ecological factors

(Bauer and Federle 2009; Wang et al. 2009). In *Nepenthes* pitchers, the waxy surface is almost unwettable (Figs. 1, 9J, K), whereas the peristome-lid (Figs. 1, 9A-I) and digestive surfaces (Figs. 1, 9L, M) are wettable. The thermographic profiles are displaying the low temperature nighttime zones of peristome-lid portions in *Nepenthes* pitchers and emphasizing their significance in wettability and prey capture.

Nepenthes pitchers *versus* thermogenic flowers

Thermoregulation is a trait usually attributed to endothermic animals (mammals, birds). Thermogenesis is also displayed by several plant families; it enhances fragrance production, volatility and has a functional role of providing an energy reward for insect visitors (Seymour et al. 2003; Seymour et al. 2009). Thermogenic flowers are capable of sensing external temperature changes and modulating the rate of heat production controlled by cellular enzymatic processes; and they maintain constant temperature across a wide range of ambient temperatures (Seymour 2001; Seymour et al. 2003). Sacred lotus (*Nelumbo nucifera*), Amazon water lily (*Victoria amazonica*) and *Philodendron selloum* (Araceae) are classic examples of thermogenic plants (Seymour 1997; Lamprecht et al. 1998; Seymour and Matthews 2006). The capacity for heat production varies markedly among thermogenic species, ranging from 2 to 3 degrees to almost 40 degrees above ambient, for a few hours to days and even weeks (for example, inflorescences of *P. selloum*) (Lamprecht et al. 1998; Lamprecht et al. 2002; Grant et al. 2008). Pollinators are able to detect floral thermal patterns, and they prefer this 'heat reward' (Seymour 1997; Grant et al. 2008; Harrap et al. 2017). Rands and Whitney (2008) stated that temperature receptors in bee's antennae are sensitive to temperature variations, and can sense differences as low as 0.25°C. Thermogenesis may also prevent low temperature damage or ensure an optimum temperature for floral development (Grant et al. 2008).

V. amazonica (Fig. 2) is an edible, rhizomatous aquatic plant (Lim et al. 2016) native to SE Colombia, N & E Bolivia and Guyana. Pollination of *V. amazonica* is known to be associated with *Cyclocephala* beetles, and it is known to display floral thermogenicity (Seymour and Matthews 2006). Seymour and Matthews (2006) reported that on the first

day the mean floral chamber temperature in *V. amazonica* peaked at 34.7ºC at 1940 h and gradually fell during the night to a mean low of 29.3ºC at 0620 h; the mean ambient air temperatures decreased from 25.2 to 23.5ºC. The grand mean floral chamber temperature was 31.7ºC between sunset at 1844 h and sunrise at 0641 h (mean air temperature 24.1ºC). On the second night, mean floral temperature was lower at the same times of day, decreasing from 30.2ºC (1940 h) to 28.3ºC (0620 h); and the mean air temperature decreased from 25.1 to 23.9ºC. During the day, temperatures in unhooded flowers rose in response to rising ambient temperature, but remained considerably below it during the hottest part of the day, due to evaporative cooling (Seymour and Matthews 2006). In our infrared thermographic measurements, the base of the floral lobes of *V. amazonica* (sp2) remained hotter almost throughout the 48 h measurements. The flower base (sp1) and top (sp3) petals mostly remained below ambient temperature (Figs. 2, 7, S4, Tables S13, S14). The floral chamber temperatures were not measured in thermographic experiments.

N. nucifera is native to Ukraine, N Iran, far East Russia to tropical Asia and N, NE Australia; its flower is considered as a classic example of beetle pollination (Bernhardt 2000; Davis et al. 2008; Li and Huang 2009) (Fig. 2). One wild flower of *N. nucifera* produces around 1 million pollen grains, which is an exceptionally high number for a bee-pollinated species (Li and Huang 2009). *N. nucifera* is known to regulate flower temperatures between 30 and 36°C for up to 4 days in environments between 10 and 45°C (Lamprecht et al. 1998; Seymour 1997; Seymour and Schultze-Motel 1998; Li et al. 2022). In this study (Figs. 2, 7, S5, Table S15), the central floral portions (sp3) of *N. nucifera* remained hotter almost throughout the day; sp3 remained 7.7ºC hotter (compared to ambient atmospheric temp.) at 8 am. The inner (sp2) and outer (sp1) floral lobes remained hotter (compared to ambient atmospheric temp.) only in the daytimes (10 am to 3 pm) and (11 am to 4 pm), respectively (Figs. 7C, S5, Table S15).

The non-thermogenic (control) flowers *S. cannifolium*, *S. wallisii*, *A. commutatum*, and two rose hybrid(s) (Fig. 2) remained below the atmospheric temperatures throughout the

day, except rarely their zones emerged above ambient in the midday (Figs. 2, S7, Tables S16-S20).

Recent studies demonstrated that bees are sensitive to floral humidity (von Arx et al. 2012; von Arx 2013; Harrap et al. 2021; Dahake et al. 2022), and on a much larger scale, variations in environmental humidity influence insect behavioral responses (Chown et al. 2011; Enjin 2017). Further, bee responses demonstrated that floral temperature patterns can signal reward location, and therefore they function as floral guides (Harrap et al. 2017; Harrap et al. 2020). In thermogenic plants, floral zones retain temperatures higher than the ambient, thus provides a 'thermal reward' and help the pollinator to 'fly away'. Floral thermogenesis is controlled by enzymatic processes. In contrast, the trapping zones in *Nepenthes* pitchers achieve low temperatures by physical (peristome microstructures), chemical (nectar) and ecological (humidity, rain) factors. Both (pollination and prey capture) are contrasting 'life' and 'death' scenarios in nature. The interactions of insects and other arthropods to these pitcher surfaces are to be further investigated considering their sensitivities towards surface (peristome) temperature, humidity, nectar composition and CO2 emissions.

Prey capture strategies

We reported UV induced fluorescence emissions as visual cues from the prey capture spots of *Nepenthes* and other carnivorous plants (Kurup et al. 2013). The intensity of the blue emission is coinciding with the pitcher growth, linking it to prey capture; blue signal intensity faded away in old pitchers (Kurup et al. 2013). Further, HPTLC profiles of *N. khasiana* peristome extracts displayed multiple blue fluorescent bands (Fig. 10). Confocal microscopy of *N. khasiana* peristome cross sections displayed vivid blue emissions and its surface microstructures (Fig. 8). Several studies are now available demonstrating the role of fluorescence signaling in plants and animals (Baby et al. 2013; Taboada et al. 2017; Mori et al. 2018; Kohler et al. 2019; Ben-Zvi et al. 2022). In plants, visual cues are usually observed in animal-pollinated species.

Scanning electron micrographs showed nectaries at the inner sides of *N. khasiana* pitcher peristomes (Fig. 9F-I). Dry nectar deposits on *N. khasiana* lids were observed in direct SEM images (Fig. 9D-E), along with glands (Fig. 9A-C). Nectar in *Nepenthes* pitcher lids was reported by several authors (Bauer et al. 2016); it is the primary reward in mutualistic interactions reported in several Bornean species (Clarke et al. 2009; Bauer et al. 2016). Peristome microstructures and modified stomata are also visible in SEM images (Fig. 9F-K). We recently demonstrated *Nepenthes* (unopened) pitchers as CO2-enriched cavities, and this as the reason for the modification of stomata (single guard cell) within the pitchers (Fig. 9J-K) (Baby et al. 2017). CO2 is slowly released from open *Nepenthes* pitchers as a cue attracting insects; they also help keeping the pitcher fluid acidic, enabling the functioning of the digestive enzymes (Baby et al. 2017). *Datura wrightii*, native to south West and south Central USA and north Mexico, has fragrant flowers which are pollinated by hawkmoths at night. Its nectar-rich flowers when open at dusk releases above-ambient levels of CO2, but floral CO2 diminishes rapidly after visitation by *Manduca sexta* moths, whereas floral scent and visual display persist for hours after nectar removal. Elevated CO2 temporarily functions as the most accurate nectar guide for foraging *Manduca* moths in *Datura* flowers, whereas visual and olfactory signals continue guiding hawkmoths to these flowers (Thom et al. 2004; Guerenstein et al. 2004; Goyret et al. 2008; von Arx et al. 2012). Similarly, above ambient emission of CO2 from open *Nepenthes* pitchers is an indicator guiding preys towards the nectar (and the trap) (Baby et al. 2017).

Thermogenic plants rely on alternative oxidase (AOX), which is an enzyme in the mitochondria organelle and is a part of the electron transport chain. The reduction of mitochondrial redox potential by AOX increases unproductive respiration. This metabolic process creates an excess of heat which warms thermogenic tissue or organs (Vanlerberghe 2013). Pavlovič and Kocáb (2022) reported high amount of AOX in *Nepenthes* pitcher traps, and discussed its roles in prey attraction, homeostasis of reactive oxygen species, balance between photosynthetic light-dark reactions, digestive physiology and nutrient assimilation. The roles of alternative oxidases in thermogenic and pitcher plants are to be further investigated in the context pollination and prey capture.

SEM images of inner side of *N. khasiana* pitcher lids showed glands (Fig. 9A-C), similarly, liquid zone displayed digestive glands (Fig. 9L, M). On prey capture, studies also detected the release of antifungal metabolites (naphthoquinones) to *N. khasiana* pitcher fluids (Raj et al. 2011; Rahman-Soad et al. 2021). From top to bottom, the morphology and structure of the leaf-evolved traps (pitchers) in *Nepenthes* are adapted or equipped towards carnivory (Thorogood et al. 2018).

Conclusions

Nepenthes pitcher peristome and peristome-lid interface acquire low temperatures due to their surface microstructures, secreted nectar and ecological features such as humidity and rain fall. These low temperatures are achieved in evening-night-morning periods of the day when the humidity is high. *Nepenthes* pitchers on opening expose their peristomes displaying the (i) blue fluorescence, (ii) nectaries at their inner sides, (iii) peristome surface with continuous, directional water transport, (iv) peristome and peristome-lid interface which record the lowest temperatures, (v) releases CO2 to the atmosphere, (v) exposes toxic metabolites secreted through the nectar and (vi) releases antifungal metabolites into the pitcher fluid. The peristome epidermal microstructures make it a hydrophilic wettable surface. Wet peristome surface at high humidity (low temperatures) at the night and/or on rainfall mimics ‘aquaplaning’, prevents insect foothold to its surface and makes it extremely slippery.

This study demonstrates thermal patterns in *Nepenthes* pitchers supporting their evolutionary strategy of prey capture. The prey capturing zones (peristome, lid and their intersection) are colder compared to other pitcher regions and ambient temperatures. In contrast, in thermogenic flowers, floral portions are hotter, providing the thermal requirements for the pollinator to fly away and transfer the pollen. Prey capture and pollination are two contrasting natural phenomena, and this study evidences opposite thermal patterns in pitcher and floral organs supporting these events and thereby survival of these plants. Both these phenomena acquired similar attributes (nectar, visual cues, CO2, volatiles) through evolution. *Nepenthes* also displays floral traits (pollen, nectar, volatiles) in addition to prey trapping (pitcher) features. Low temperature at the peristome

and lid portions of *Nepenthes* pitchers is contradicting the thermal reward offered by thermogenic flowers, but the capture (rates) by these traps are significant favoring the influence of the visual, gaseous and other cues in prey attraction.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data are provided as Supporting Information.

Acknowledgements

We are grateful to Dr. K.J. Lathan Kumar and Dr. R. Raj Vikraman, Garden Management Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram for providing us the plant specimens and facilities for the field studies.

Funding

This study was supported by Science and Engineering Research Board (SERB), Government of India [grant number CRG/2019/000131] and the Kerala State Council for Science Technology and Environment (KSCSTE), Government of Kerala [grant number P-07A/JNTBGRI].

Author contributions

SB conceptualized and designed the research, acquired funding; GBS, AJJ performed experiments; SB, GBS, AJJ analyzed the data and prepared the figures; AAZ provided field support; SB wrote the manuscript with contributions from all the authors; All authors read and approved the manuscript.

Appendix A. Supporting Information

Data used in this paper are available in the electronic Supporting Information.

Table S1 to S12. Thermogenic patterns of unopen and open *N. khasiana* pitchers.

Tables S13 and S14. Thermogenic patterns of *V. amazonica*.

Table S15. Thermogenic patterns of *N. nucifera*.

Tables S16 to S20. Thermographic profiles of non-thermogenic flowers (A) *S. cannifolium*; (B) *S. wallisii*; (C) *A. commutatum* and (D) rose hybrid 1.

Fig. S1. Thermographic profiles of *Nepenthes* hybrids: (A) hybrid 1 unopen pitcher; (B) hybrid 2 unopen pitcher; (C) hybrid 1 open pitcher; (D) hybrid 2 open pitcher; (E) hybrid 3 open pitcher; (F) hybrid 4 open pitcher.

Fig. S2. Nighttime duration (black shaded) in unopen *N. khasiana* pitcher spots (P1U to P6U) at > 3 degrees below the atmospheric temperature.

Fig. S3. Nighttime duration (black shaded) in *N. khasiana* open pitcher spots (P1O to P6O) at > 3 degrees below the atmospheric temperature.

Fig. S4. Thermographic profiles of *V. amazonica* (A) flower bud above the level of water (1600); (B) flower bloomed, three clear spots (sp1, sp2, sp3) visible (1900); (C & D) flower showing three spots but intensity of sp2 decreased (0200 & 0600); (E) flower is slightly pink and started to fold (1000); (F) flower in completely pink, partially opened, sp2 is visible but with low intensity (1600); (G) flower fully opened and sp2 is of low intensity (1800); (H) flower top is more opened and a hot central portion is visible (1900); (I) flower is partially floating in water, central region is visible (0200); (J) flower is partially floating, central region visible with low intensity (0600); (K) flower floats above water not spot differentiation visible (1000); (L) flower almost immersed in water with some portions floating above, nearly dead state (1600).

Fig. S5. Thermographic profiles of *N. nucifera* (A) fully bloomed flower in open pond, three regions clearly spotted (1800); (B) flower partially closed, three regions not clearly visible (2200); (C) flower partially opened, regions begins to appear (0600); (D) flower completely opened, all regions clearly spottable (1000); (E) flower, totally opened and is moisture free (1100); (F) flower began to close, all regions visible (1800).

Fig. S6. Thermographic profiles of non-thermogenic flowers (A) *S. cannifolium*; (B) *S. wallisii*; (C) *A. commutatum* and (D) rose hybrid 1.

Fig. S7. Thermographic patterns of control flowers (A) *S. cannifolium*, (B) *S. wallisii*, (C) *A. commutatum*, and (D, E) two rose hybrid(s) in field conditions; (dotted line: ambient

atm. temp., black: sp1, pink: sp2; blue: sp3; X-axis (circular) (A-E): 1-24 are 24 h time at 1 h measurement intervals starting 6 pm to 5 pm, next day (Tables S16-S20); Y-axis: temperature in degrees).

References

von Arx M, Goyret J, Davidowitz G, Raguso RA. 2012. Floral humidity as a reliable sensory cue for profitability assessment by nectar-foraging hawkmoths. Proc Natl Acad Sci USA 109:9471-9476, https://doi.org/10.1073/pnas.1121624109.

von Arx M. 2013. Floral humidity and other indicators of energy rewards in pollination biology. Commun Integr Biol 6:e22750, https://doi.org/10.4161/cib.22750.

Baby S, Johnson AJ, Govindan B, Lukose S, Gopakumar B, Koshy KC. 2013. UV induced visual cues in grasses. Sci Rep 3:2738, https://doi.org/10.1038/srep02738.

Baby S, Johnson AJ, Zachariah EJ, Hussain AA. 2017. *Nepenthes* pitchers are CO2-enriched cavities, emit CO2 to attract preys. Sci Rep 7:11281, https://doi.org/10.1038/s41598-017-11414-7.

Bauer U, Bohn HF, Federle W. 2008. Harmless nectar source or deadly trap: *Nepenthes* pitchers are activated by rain, condensation and nectar. Proc R Soc Lond B Biol Sci 275:259-265, doi: 10.1098/rspb.2007.1402.

Bauer U, Federle W. 2009. The insect-trapping rim of *Nepenthes* pitchers: surface structure and function. Plant Signal Behav 4:1019-1023, https://doi.org/10.4161/psb.4.11.9664.

Bauer U, Rembold K, Grafe TU. 2016. Carnivorous *Nepenthes* pitcher plants are a rich food source for a diverse vertebrate community. J Nat Hist 50:483-495.

Ben-Zvi O, Lindemann Y, Eyal G, Loya Y. 2022. Coral fluorescence: a prey-lure in deep habitats. Commun Biol 5:537. https://doi.org/10.1038/s42003-022-03460-3.

Bernhardt P. 2000. Convergent evolution and adaptive radiation of beetle-pollinated angiosperms. Plant Syst Evol 222:293-320. https://doi.org/10.1007/BF00984108.

Bohn HF, Federle W. 2004. Insect aquaplaning: *Nepenthes* pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. Proc Natl Acad Sci USA 101:14138-14143, https://doi.org/10.1073/pnas.040588510.

Chen H, Zhang P, Zhang L, Liu H, Jiang Y, Zhang D, Han Z, Jiang L. 2016. Continuous directional water transport on the peristome surface of *Nepenthes alata*. Nature 532:85-89, https://doi.org/10.1038/nature17189.

Chown SL, Sørensen JG, Terblanche JS. 2011. Water loss in insects: an environmental change perspective. J Insect Physiol 57:1070-1084, https://doi.org/10.1016/j.jinsphys.2011.05.004.

Clarke CM, Bauer U, Lee CIC, Tuen AA, Rembold K, Moran JA. 2009. Tree shrew lavatories: a novel nitrogen sequestration strategy in a tropical pitcher plant. Biol Lett 5:632-635, https://doi.org/10.1098/rsbl.2009.0311.

Clarke C, Moran JA. 2016. Climate, soils and vicariance-their roles in shaping the diversity and distribution of *Nepenthes* in Southeast Asia. Plant Soil 403:37-51, https://doi.org/10.1007/s11104-015-2696-x.

Dahake A, Jain P, Vogt CC, Kandalaft W, Stroock AD, Raguso RA. 2022. A signal-like role for floral humidity in a nocturnal pollination system. Nature Commun 13:7773, https://doi.org/10.1038/s41467-022-35353-8.

Davis CC, Endress PK, Baum DA. 2008. The evolution of floral gigantism. Curr Opin Plant Biol 11: 49-57, https://doi.org/10.1016/j.pbi.2007.11.003.

Dyer AG, Whitney HM, Arnold SE, Glover BJ, Chittka L. 2006. Bees associate warmth with floral colour. Nature 442:525-525, https://doi.org/10.1038/442525a.

Enjin A. 2017. Humidity sensing in insects-from ecology to neural processing. Curr Opin Insect Sci 24:1-6, https://doi.org/10.1016/j.cois.2017.08.004.

Goyret J, Markwell PM, Raguso RA. 2008. Context-and scale-dependent effects of floral CO2 on nectar foraging by *Manduca sexta*. Proc Natl Acad Sci USA 105:4565-4570, https://doi.org/10.1073/pnas.0708629105.

Grant NM, Miller RE, Watling JR, Robinson SA. 2008. Synchronicity of thermogenic activity, alternative pathway respiratory flux, AOX protein content, and carbohydrates in receptacle tissues of sacred lotus during floral development. J Exp Bot 59:705-714, https://doi.org/10.1093/jxb/erm333.

Gray LK, Clarke C, Wint GW, Moran JA. 2017. Potential effects of climate change on members of the Palaeotropical pitcher plant family Nepenthaceae. PloS One 12:e0183132, https://doi.org/10.1371/journal.pone.0183132.

Guerenstein PG, Yepez EA, van Haren J, Williams DG, Hildebrand JG. 2004. Floral CO2 emission may indicate food abundance to nectar-feeding moths. Naturwissenschaften 91:329-333, https://doi.org/10.1007/s00114-004-0532-x.

Harrap MJ, Hempel de Ibarra N, Knowles HD, Whitney HM, Rands SA. 2021. Bumblebees can detect floral humidity. J Exp Biol 224:jeb240861, https://doi.org/10.1242/jeb.240861.

Harrap MJ, Hempel de Ibarra N, Whitney HM, Rands SA. 2020. Floral temperature patterns can function as floral guides. Arthropod Plant Interact 14:193-206, https://doi.org/10.1007/s11829-020-09742-z.

Harrap MJ, Rands SA, Hempel de Ibarra N, Whitney HM. 2017. The diversity of floral temperature patterns, and their use by pollinators. Elife 6:e31262, https://doi.org/10.7554/eLife.31262.

Hsu CP, Lin YM, Chen PY. 2015. Hierarchical structure and multifunctional surface properties of carnivorous pitcher plants *Nepenthes*. JOM 67:744-753, https://doi.org/10.1007/s11837-015-1349-0.

Kanie S, Miura D, Jimi N, Hayashi T, Nakamura K, Sakata M, Mitani Y. 2021. Violet bioluminescent *Polycirrus* sp. (Annelida: Terebelliformia) discovered in the shallow coastal waters of the Noto Peninsula in Japan. Sci Rep 11:19097, https://doi.org/10.1038/s41598-021-98105-6.

Kohler AM, Olson ER, Martin JG, Anich PS. 2019. Ultraviolet fluorescence discovered in New World flying squirrels (Glaucomys). J Mammal 100:21-30, https://doi.org/10.1093/jmammal/gyy177.

Kurup R, Johnson AJ, Sankar S, Hussain AA, Kumar CS, Sabulal B. 2013. Fluorescent prey traps in carnivorous plants. Plant Biol 15:611-615, https://doi.org/10.1111/j.1438-8677.2012.00709.x.

Lamprecht I, Seymour RS, Schultze-Motel P. 1998. Direct and indirect calorimetry of thermogenic flowers of the sacred lotus, *Nelumbo nucifera*. Thermochim Acta 309:5-16, https://doi.org/10.1016/S0040-6031(97)00423-1.

Lamprecht I, Schmolz E, Blanco L, Romero CM. 2002. Infrared thermography of a tropical water plant. Thermol Int 12:91-99.

Li JK, Huang SQ. 2009. Effective pollinators of Asian sacred lotus (*Nelumbo nucifera*): contemporary pollinators may not reflect the historical pollination syndrome. Ann Bot 104:845-851, https://doi.org/10.1093/aob/mcp173.

Lim TK. 2016. *Edible Medicinal and Non-Medicinal Plants. Modified Stems, Roots, Bulbs*. Springer International Publishing, Cham, Switzerland, Vol. 11, https://doi.org/10.1007/978-3-319-26065-5.

Li R, Li J, Wang S, Wang R. 2022. Novel insights into floral thermogenesis: *In vivo* analyses of mitochondrial dynamics in *Nelumbo nucifera* flowers. Int J Mol Sci 23:11950, https://doi.org/10.3390/ijms231911950.

Merlot S, Mustilli AC, Genty B, North H, Lefebvre V, Sotta B, Giraudat J. 2002. Use of infrared thermal imaging to isolate *Arabidopsis* mutants defective in stomatal regulation. Plant J 30:601-609, https://doi.org/10.1046/j.1365-313X.2002.01322.x.

Miguel S, Hehn A, Bourgaud F. 2018. *Nepenthes*: State of the art of an inspiring plant for biotechnologists. J Biotechnol 265:109-115, https://doi.org/10.1016/j.jbiotec.2017.11.014.

Moran JA, Clarke CM. 2010. The carnivorous syndrome in *Nepenthes* pitcher plants: current state of knowledge and potential future directions. Plant Signal Behav 5:644-648, https://doi.org/10.4161/psb.5.6.11238.

Moran JA, Gray LK, Clarke C, Chin L. 2013. Capture mechanism in Palaeotropical pitcher plants (Nepenthaceae) is constrained by climate. Ann Bot 112:1279-1291, https://doi.org/10.1093/aob/mct195.

Mori S, Fukui H, Oishi M, Sakuma M, Kawakami M, Tsukioka J, Hirai N 2018. Biocommunication between plants and pollinating insects through fluorescence of pollen and anthers. J Chem Ecol 44:591-600, https://doi.org/10.1007/s10886-018-0958-9.

Pavlovič A, Masarovičová E, Hudák J. 2007. Carnivorous syndrome in Asian pitcher plants of the genus *Nepenthes*. Ann Bot 100:527-536, https://doi.org/10.1093/aob/mcm145.

Pavlovič A, Kocáb O. 2022. Alternative oxidase (AOX) in the carnivorous pitcher plants of the genus *Nepenthes*: what is it good for? Ann Bot 129:357-365, https://doi.org/10.1093/aob/mcab151.

Rahman-Soad A, Dávila-Lara A, Paetz C, Mithöfer A. 2021. Plumbagin, a potent naphthoquinone from *Nepenthes* plants with growth inhibiting and larvicidal activities. Molecules 26:825, https://doi.org/10.3390/molecules26040825.

Raj G, Kurup R, Hussain AA, Baby S. 2011. Distribution of naphthoquinones, plumbagin, droserone, and 5-O-methyl droserone in chitin-induced and uninduced *Nepenthes khasiana*: molecular events in prey capture. J Exp Bot 62:5429-5436, https://doi.org/10.1093/jxb/err219.

Rands SA, Whitney HM. 2008. Floral temperature and optimal foraging: is heat a feasible floral reward for pollinators? PLoS One 3:e2007, doi: 10.1371/journal.pone.0002007.

Seymour RS. 1997. Plants that warm themselves. Sci Am 276:104-109.

Seymour RS. 2001. Biophysics and physiology of temperature regulation in thermogenic flowers. Biosci Rep 21:223-236, https://doi.org/10.1023/A:1013608627084.

Seymour RS, Ito Y, Onda Y, Ito K. 2009. Effects of floral thermogenesis on pollen function in Asian skunk cabbage *Symplocarpus renifolius*. Biol Lett 5:568-570, doi: 10.1098/rsbl.2009.0064.

Seymour RS, Matthews PG. 2006. The role of thermogenesis in the pollination biology of the Amazon waterlily *Victoria amazonica*. Ann Bot 98:1129-1135, https://doi.org/10.1093/aob/mcl201.

Seymour RS, White CR, Gibernau M. 2003. Heat reward for insect pollinators. Nature 426:243-244, https://doi.org/10.1038/426243a.

Taboada C, Brunetti AE, Pedron FN, Carnevale Neto F, Estrin DA, Bari SE, Faivovich J. Naturally occurring fluorescence in frogs. Proc Natl Acad Sci USA 114:3672-3677, https://doi.org/10.1073/pnas.1701053114.

Tattersall GJ. 2016. Infrared thermography: A non-invasive window into thermal physiology. Comp Biochem Physiol A Mol Integr Physiol 202:78-98, https://doi.org/10.1016/j.cbpa.2016.02.022.

Thom C, Guerenstein PG, Mechaber WL, Hildebrand JG. 2004. Floral CO2 reveals flower profitability to moths. J Chem Ecol 30:1285-1288, https://doi.org/10.1023/B:JOEC.0000030298.77377.7d.

Thorogood CJ, Bauer U, Hiscock SJ. 2018. Convergent and divergent evolution in carnivorous pitcher plant traps. New Phytol. 217:1035-1041, https://doi.org/10.1111/nph.14879.

Usamentiaga R, Venegas P, Guerediaga J, Vega L, Molleda J, Bulnes FG. 2014. Infrared thermography for temperature measurement and non-destructive testing. Sensors 14:12305-12348, https://doi.org/10.3390/s140712305.

Vanlerberghe GC. 2013. Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signalling homeostasis during abiotic and biotic stress in plants. Int J Mol Sci 14:6805-6847, https://doi.org/10.3390/ijms14046805.

Wang L, Zhou Q. 2016. Surface hydrophobicity of slippery zones in the pitchers of two *Nepenthes* species and a hybrid. Sci Rep 6:19907, https://doi.org/10.1038/srep19907.

Wang Y, Holroyd G, Hetherington AM, Ng CKY. 2004. Seeing ‘cool’ and ‘hot’ - infrared thermography as a tool for non-invasive, high-throughput screening of *Arabidopsis* guard cell signalling mutants. J Exp Bot 55:1187-1193, https://doi.org/10.1093/jxb/erh135.

Wang L, Zhou Q, Zheng Y, Xu S. 2009. Composite structure and properties of the pitcher surface of the carnivorous plant *Nepenthes* and its influence on the insect attachment system. Prog Nat Sci 19:1657-1664, https://doi.org/10.1016/j.pnsc.2009.09.005.

Figure legends

Fig. 1. Line diagram of *N. khasiana* open pitcher with spots sp1 to sp5 marked from bottom to top. In unopen pitchers, only three spots (sp1 to sp3) were marked (measured), with sp3 as the front centre spot of the top peristome-lid interface (in closed position).

Fig. 2. Thermogenic flowers, (A) *V. amazonica,* (B) *N. nucifera* and non-thermogenic flowers, (C) *S. cannifolium,* (D) *S. wallisii*, (E) *A. commutatum* and (F) rose hybrid.

Fig. 3. Infrared thermographic profiles of six unopen *N. khasiana* pitchers in field conditions; (A) P1U, (B) P2U, (C) P3U, (D) P4U, (E) P5U, (F) P6U. P1U to P3U & P4U to P6U were recorded in January 2019 and March 2021, respectively (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3; spots are as labelled in Fig. 1; X-axis (circular) (A-F): 1-24 are 24 h time at 1 h measurement intervals starting 6 pm to 5 pm, next day (Tables S1-S6); Y-axis: temperature in degrees).

Fig. 4. Infrared thermographic profiles of six open *N. khasiana* pitchers in field conditions; (A) P1O, (B) P2O, (C) P3O, (D) P4O, (E) P5O, (F) P6O. P1O to P3O & P4O to P6O were recorded in January 2019 and March 2021, respectively (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3, red: sp4, green: sp5; spots are as labelled in Fig. 1; X-axis (circular) (A-F): 1-24 are 24 h time at 1 h measurement intervals starting 6 pm to 5 pm, next day (Tables S7-S12); Y-axis: temperature in degrees).

Fig. 5. *N. khasiana* pitcher opening monitored by infrared thermography, (A) 12.30 am, (B) 4.30 am, (C) 5.30 am, (D) 6.30 am, (E) 7.30 am, (F) 8.30 am, (G) 9.30 am, (H) 10.30 am, (I) 11.30 am, measurements on March 28, 2018.

Fig. 6. Infrared thermographic profile of *N. khasiana* pitcher in controlled laboratory conditions; (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3, red: sp4, green: sp5; spots are as labelled in Fig. 1; X-axis: measurement data points (13) in 30 min duration).

Fig. 7. Thermographic patterns of (A) *V. amazonica*, 4 pm to 10 am (18 h), January 2019; (B) *V. amazonica*, 4 pm to 3 pm (48 h), March 2021; (C) *N. nucifera*, 6 pm to 5 pm (24 h), March 2021; (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3; X-axis (circular) (A): 1-19, time at 1 h measurement intervals starting 4 pm to 10 am, next day (Table S13); X-axis (circular) (B): 1-48, time at 1 h measurement intervals starting 4 pm

to 3 pm, 48 h (Table S14); X-axis (circular) (C): 1-24, time at 1 h measurement intervals starting 6 pm to 5 pm, 24 h (Table S15); Y-axes (A-C): temperature in degrees).

Fig. 8. Confocal microscopy of *N. khasiana* peristome cross section with visible microstructures, (A) lateral view, (B) top view.

Fig. 9. SEM pictures of various regions of *N. khasiana* pitchers, (A) lid, inner side; (B) lid, inner side, gland (expansion); (C) lid, inner side, gland (expansion); (D) direct SEM, lid, inner side, nectar deposition; (E) direct SEM, lid, inner side, nectar deposition (expansion); (F) peristome, nectaries at the inner side; (G) peristome, nectary (expansion); (H) direct SEM, peristome, nectaries at the inner side; (I) direct SEM, peristome, nectary (expansion); (J) slippery (waxy) zone; (K) slippery (waxy) zone (expansion); (L) liquid zone, glands; (M) liquid zone, glands (expansion); (N) pitcher, outer side, glands; (O) pitcher, outer side, gland (expansion).

Fig. 10. HPTLC profile of acetone extract (10 mg/mL) of *N. khasiana* peristome at 366 nm, displaying multiple blue fluorescent bands (mature pitcher peristome: track 1 10 μL, track 3 5 μL; old pitcher peristome: track 2 10 μL, track 4 5 μL).

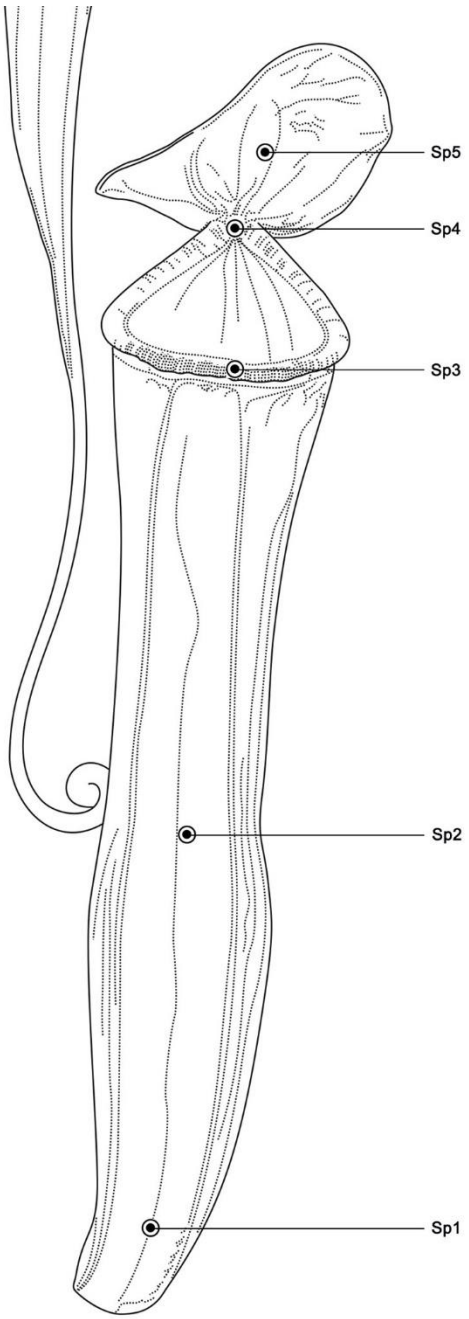

Fig. 1. Line diagram of *N. khasiana* open pitcher with spots sp1 to sp5 marked from bottom to top. In unopen pitchers, only three spots (sp1 to sp3) were marked (measured), with sp3 as the front centre spot of the top peristome-lid interface (in closed position).

Fig. 2. Thermogenic flowers, (A) *V. amazonica,* (B) *N. nucifera* and non-thermogenic flowers, (C) *S. cannifolium,* (D) *S. wallisii*, (E) *A. commutatum* and (F) rose hybrid.

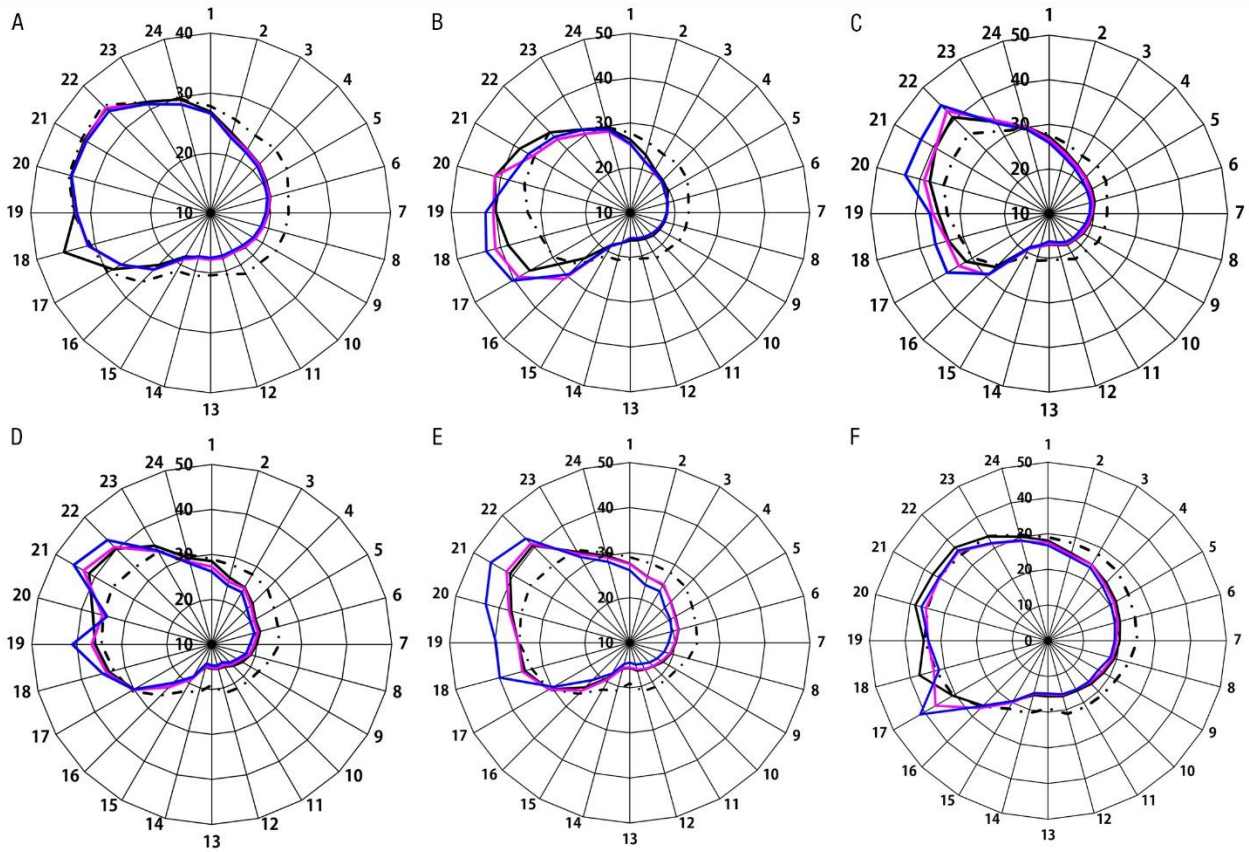

Fig. 3. Infrared thermographic profiles of six unopen *N. khasiana* pitchers in field conditions; (A) P1U, (B) P2U, (C) P3U, (D) P4U, (E) P5U, (F) P6U. P1U to P3U & P4U to P6U were recorded in January 2019 and March 2021, respectively (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3; spots are as labelled in Fig. 1; X-axis (circular) (A-F): 1-24 are 24 h time at 1 h measurement intervals starting 6 pm to 5 pm, next day (Tables S1-S6); Y-axis: temperature in degrees).

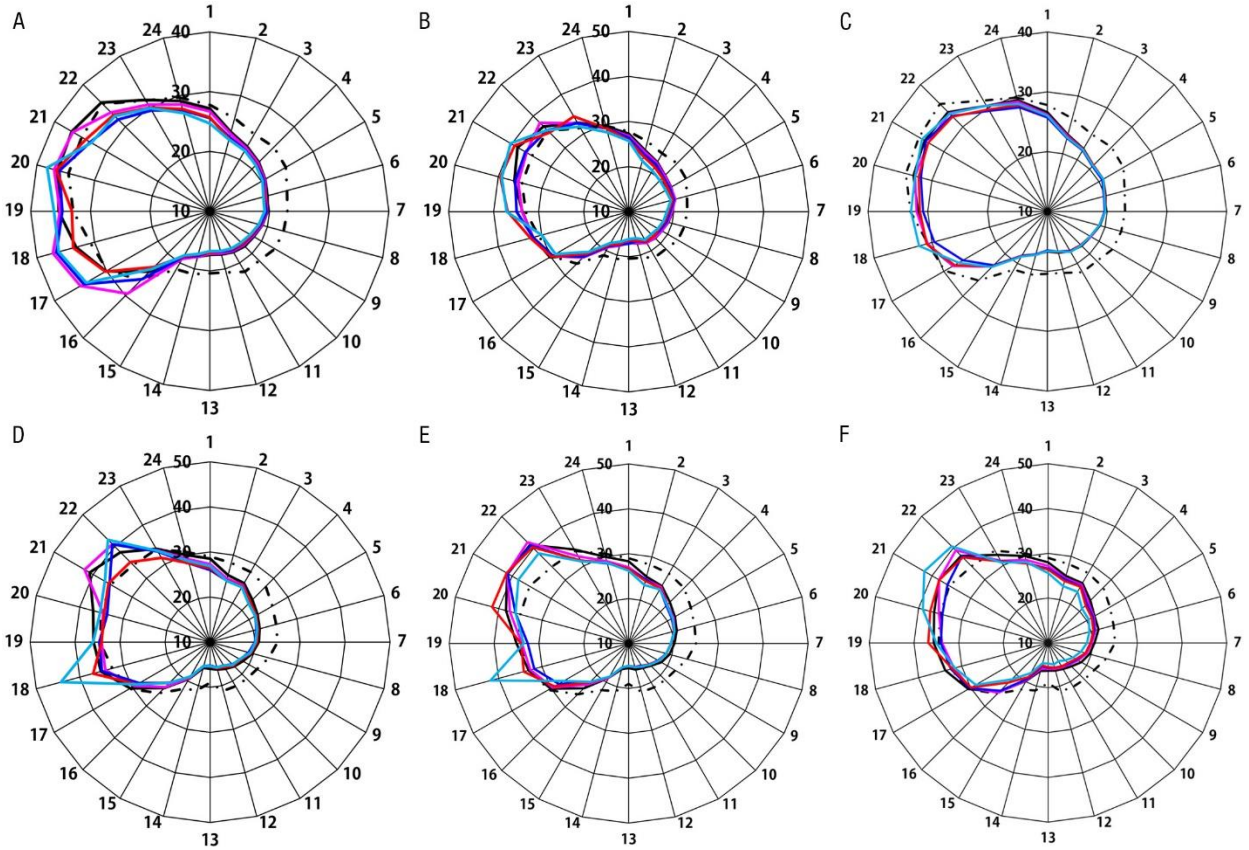

Fig. 4. Infrared thermographic profiles of six open *N. khasiana* pitchers in field conditions; (A) P1O, (B) P2O, (C) P3O, (D) P4O, (E) P5O, (F) P6O. P1O to P3O & P4O to P6O were recorded in January 2019 and March 2021, respectively (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3, red: sp4, green: sp5; spots are as labelled in Fig. 1; X-axis (circular) (A-F): 1-24 are 24 h time at 1 h measurement intervals starting 6 pm to 5 pm, next day (Tables S7-S12); Y-axis: temperature in degrees).

Fig. 5. *N. khasiana* pitcher opening monitored by infrared thermography, (A) 12.30 am, (B) 4.30 am, (C) 5.30 am, (D) 6.30 am, (E) 7.30 am, (F) 8.30 am, (G) 9.30 am, (H) 10.30 am, (I) 11.30 am, measurements on March 28, 2018.

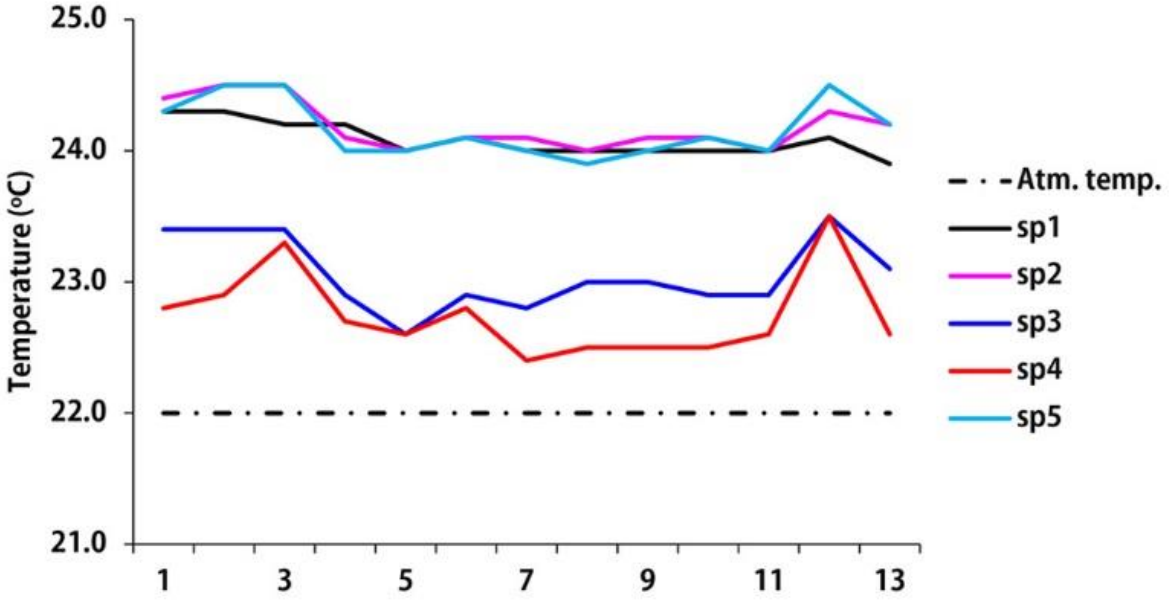

Fig. 6. Infrared thermographic profile of *N. khasiana* pitcher in controlled laboratory conditions; (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3, red: sp4, green: sp5; spots are as labelled in Fig. 1; X-axis: measurement data points (13) in 30 min duration).

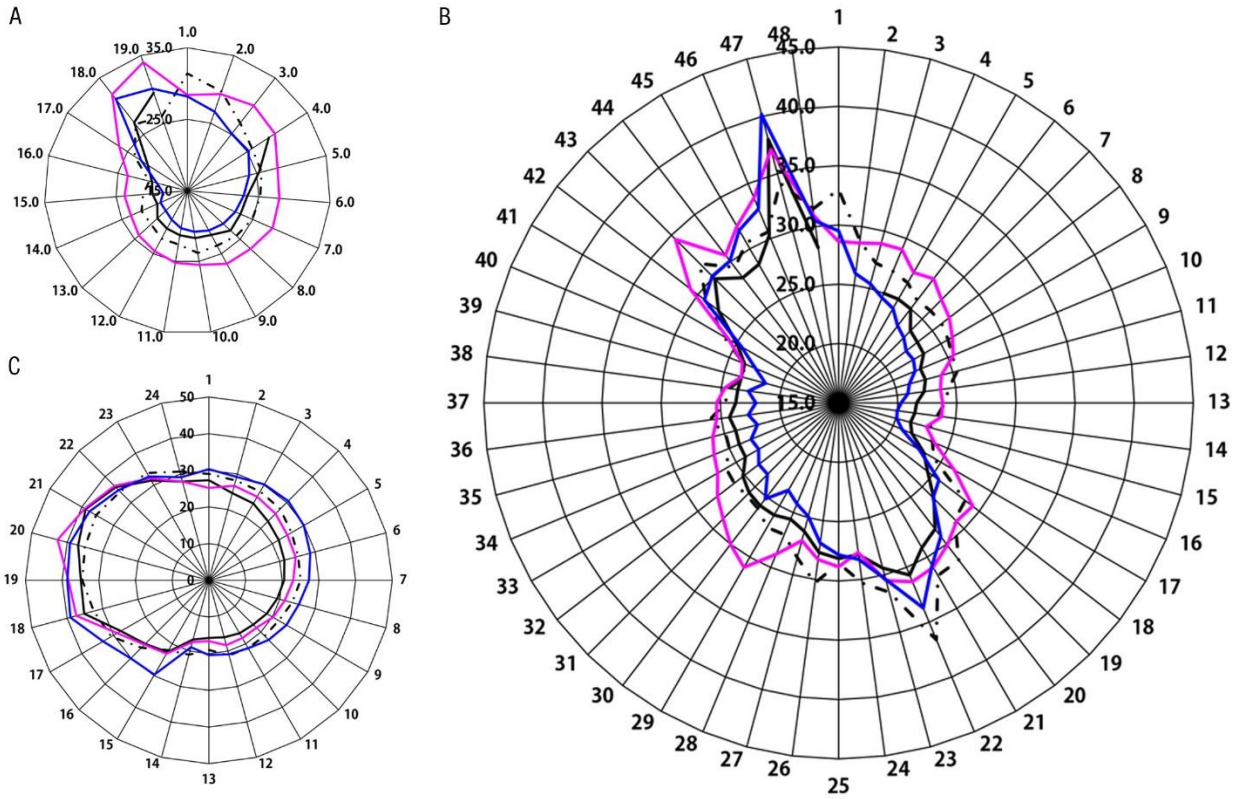

Fig. 7. Thermographic patterns of (A) *V. amazonica*, 4 pm to 10 am (18 h), January 2019; (B) *V. amazonica*, 4 pm to 3 pm (48 h), March 2021; (C) *N. nucifera*, 6 pm to 5 pm (24 h), March 2021; (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3; X-axis (circular) (A): 1-19, time at 1 h measurement intervals starting 4 pm to 10 am, next day (Table S13); X-axis (circular) (B): 1-48, time at 1 h measurement intervals starting 4 pm to 3 pm, 48 h (Table S14); X-axis (circular) (C): 1-24, time at 1 h measurement intervals starting 6 pm to 5 pm, 24 h (Table S15); Y-axes (A-C): temperature in degrees).

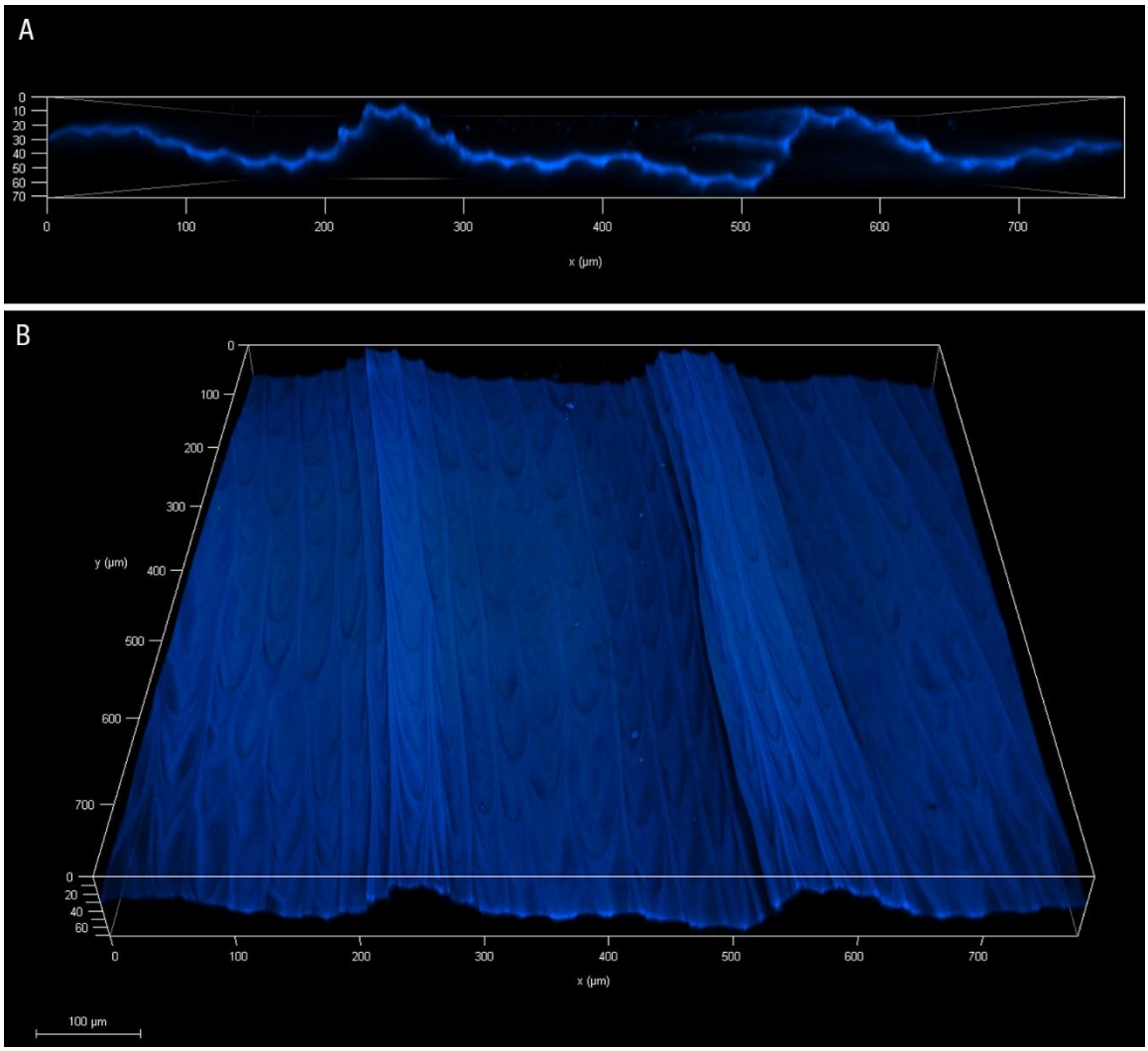

Fig. 8. Confocal microscopy of *N. khasiana* peristome cross section with visible microstructures, (A) lateral view, (B) top view.

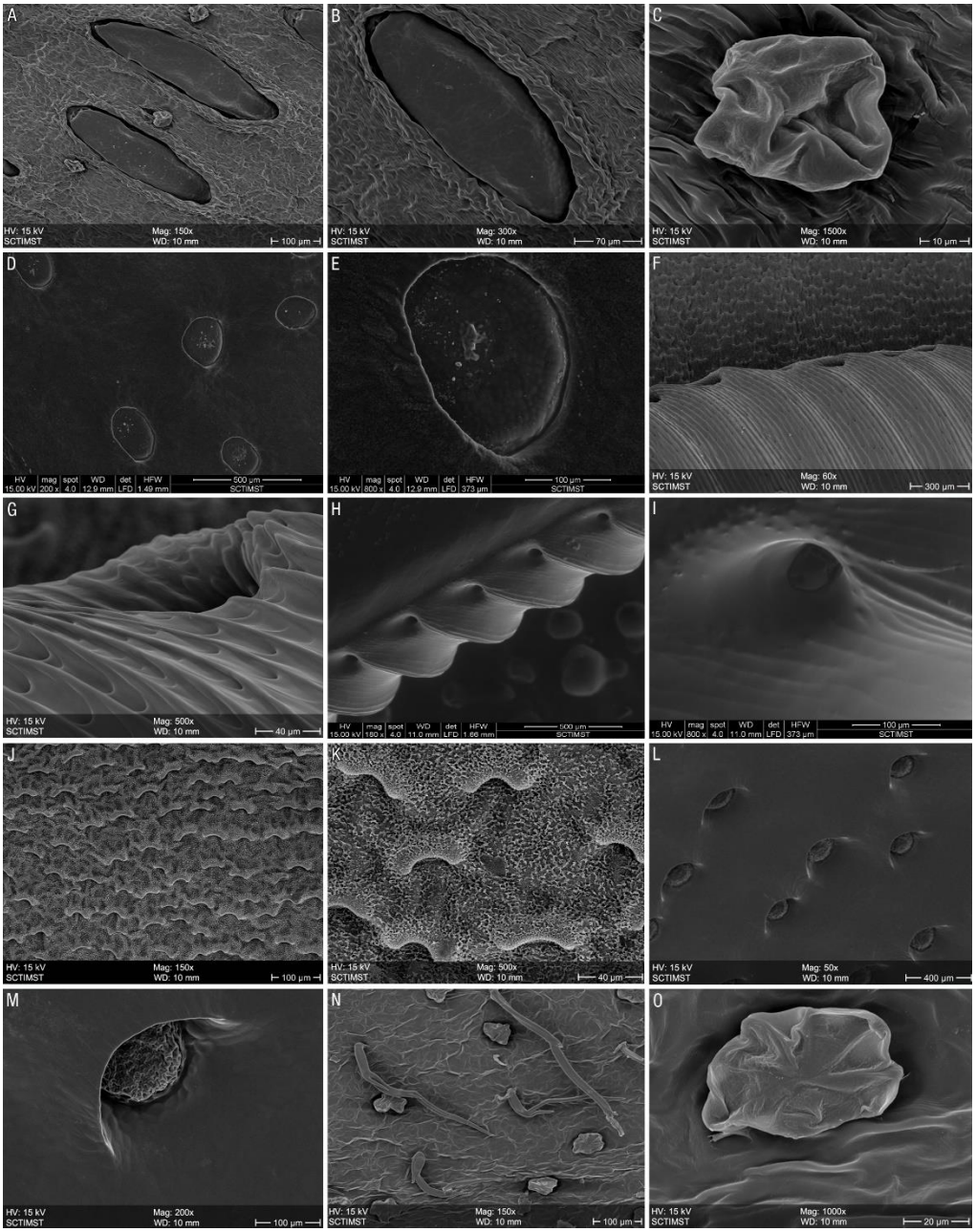

Fig. 9. SEM pictures of various regions of *N. khasiana* pitchers, (A) lid, inner side; (B) lid, inner side, gland (expansion); (C) lid, inner side, gland (expansion); (D) direct SEM, lid, inner side, nectar deposition; (E) direct SEM, lid, inner side, nectar deposition (expansion); (F) peristome, nectaries at the inner side; (G) peristome, nectary (expansion); (H) direct SEM, peristome, nectaries at the inner side; (I) direct SEM, peristome, nectary (expansion); (J) slippery (waxy) zone; (K) slippery (waxy) zone (expansion); (L) liquid zone, glands; (M) liquid zone, glands (expansion); (N) pitcher, outer side, glands; (O) pitcher, outer side, gland (expansion).

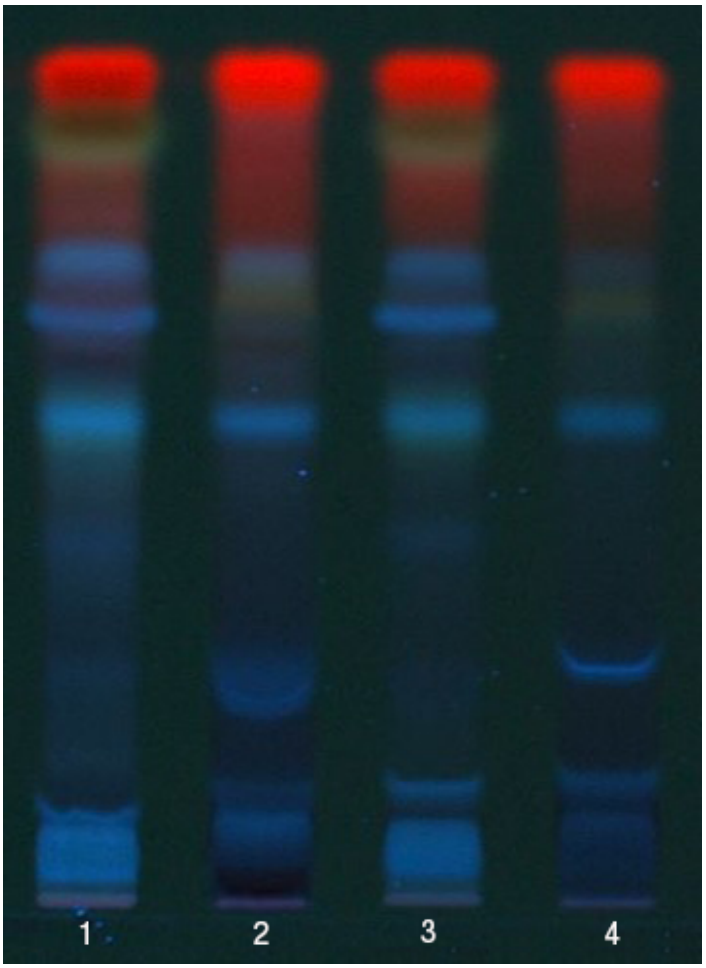

Fig. 10. HPTLC profile of acetone extract (10 mg/mL) of *N. khasiana* peristome at 366 nm, displaying multiple blue fluorescent bands (mature pitcher peristome: track 1 10 μL, track 3 5 μL; old pitcher peristome: track 2 10 μL, track 4 5 μL).

Supplementary information

Nepenthes pitchers *versus* thermogenic flowers: thermal patterns and their role in prey capture and pollination

Gokul Baburaj Sujatha*[,±], Anil John Johnson*, Abdul Azeez Hussain[¥], Sabulal Baby*[,1]

*Phytochemistry and Phytopharmacology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pacha-Palode, Thiruvananthapuram 695562, Kerala, India.

[±]University of Kerala, Thiruvananthapuram 695034, Kerala, India.

[¥]Garden Management Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pacha-Palode, Thiruvananthapuram 695562, Kerala, India

[1]E-mail: sabulal@gmail.com, sabulal@jntbgri.res.in

Abstract

Prey capture in *Nepenthes* and pollination in angiosperms are two antithetical events; one designed to trap insects and other arthropods (carnivory) and the other to transfer pollen through pollinators (reproduction). In this study, infrared thermography was extensively used to obtain thermal profiles of *Nepenthes* pitchers and thermogenic and non-thermogenic flowers in field conditions. Confocal and scanning electron microscopy were used to record peristome cross-sectional features and pitcher morphology. *N. khasiana* pitchers displayed below ambient temperatures during evening-night-morning hours (5 pm to 8-9-10 am); pitcher spots recorded lowest and highest temperatures as 14.4 (6-7 am) and 45.6^{o}C (2 pm), respectively. The average humidities in the night and day periods were 77.15% and 50.15%, respectively. The prey capturing zones in *Nepenthes* pitchers (peristome, lid and their intersection) are colder favoring prey capture, whereas in thermogenic flowers, floral portions are hotter, providing the thermal requirements for the pollinator. In thermogenic plants, floral zones assist pollination by offering a 'thermal reward' through enzymatic processes; but *Nepenthes* traps achieve lower temperature spots by physical (surface microstructures), chemical (nectar) and ecological (rain, humidity) factors. Both are contrasting 'life' and 'death' adaptations in nature.

Table S1. Thermographic data of *N. khasiana* unopen pitcher P1U.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3
1	P1U	1800	71.0	27.8	26.9	26.6	26.6
2	P1U	1900	77.0	26.1	23.8	23.3	23.0
3	P1U	2000	78.1	24.9	22.2	22.1	21.7
4	P1U	2100	77.3	25.2	21.5	21.4	21.0
5	P1U	2200	81.0	24.7	20.7	20.5	20.3
6	P1U	2300	81.0	23.4	20.3	20.2	19.8
7	P1U	2400	81.0	23.0	19.6	19.5	19.2
8	P1U	0100	81.0	22.8	19.1	19.1	18.7
9	P1U	0200	81.0	22.6	18.5	18.6	18.2
10	P1U	0300	82.0	21.7	18.1	18.0	17.6
11	P1U	0400	82.0	21.7	17.6	17.6	17.3
12	P1U	0500	83.5	20.6	17.8	17.7	17.4
13	P1U	0600	82.0	20.4	17.7	17.8	17.5
14	P1U	0700	83.0	20.9	17.6	17.8	17.5
15	P1U	0800	82.0	21.2	18.4	18.9	18.6
16	P1U	0900	79.0	26.3	22.3	23.4	23.3
17	P1U	1000	65.5	29.8	28.8	27.3	27.2
18	P1U	1100	60.6	30.7	35.3	31.2	31.1
19	P1U	1200	53.0	32.9	32.7	32.4	32.3
20	P1U	1300	42.0	34.5	34.1	34.0	34.0
21	P1U	1400	45.0	34.8	34.3	34.0	33.9
22	P1U	1500	41.5	35.4	34.6	34.8	34.0
23	P1U	1600	61.5	31.4	31.4	30.9	31.0
24	P1U	1700	67.5	29.7	29.6	28.8	28.7

Table S2. Thermographic data of *N. khasiana* unopen pitcher P2U.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3
1	P2U	1800	71.0	27.8	26.3	25.2	25.4
2	P2U	1900	77.0	26.1	23.3	22.1	21.9
3	P2U	2000	78.1	24.9	21.0	20.4	20.7
4	P2U	2100	77.3	25.2	20.2	19.8	19.9
5	P2U	2200	81.0	24.7	19.7	19.0	18.9
6	P2U	2300	81.0	23.4	18.4	18.6	18.7
7	P2U	2400	81.0	23.0	18.3	18.2	18.1
8	P2U	0100	81.0	22.8	17.9	17.7	17.7
9	P2U	0200	81.0	22.6	17.5	17.1	17.2
10	P2U	0300	82.0	21.7	17.2	16.8	16.7
11	P2U	0400	82.0	21.7	16.7	16.3	16.3
12	P2U	0500	83.5	20.6	16.4	15.9	16.0
13	P2U	0600	82.0	20.4	16.2	15.7	15.7
14	P2U	0700	83.0	20.9	17.0	17.0	17.0
15	P2U	0800	82.0	21.2	18.3	18.5	18.6
16	P2U	0900	79.0	26.3	24.4	30.9	29.5
17	P2U	1000	65.5	29.8	35.9	38.8	40.4
18	P2U	1100	60.6	30.7	38.2	41.2	43.3
19	P2U	1200	53.0	32.9	40.0	40.6	42.3
20	P2U	1300	42.0	34.5	41.3	41.3	38.1
21	P2U	1400	45.0	34.8	38.5	35.2	36.2
22	P2U	1500	41.5	35.4	35.3	33.1	33.9
23	P2U	1600	61.5	31.4	31.4	30.3	31.3
24	P2U	1700	67.5	29.7	29.6	28.7	29.2

Table S3. Thermographic data of *N. khasiana* unopen pitcher P3U.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (^{o}C)	sp1	sp2	sp3
1	P3U	1800	71.0	27.8	27.2	26.6	25.9
2	P3U	1900	77.0	26.1	24.4	23.9	23.1
3	P3U	2000	78.1	24.9	22.6	22.3	21.6
4	P3U	2100	77.3	25.2	21.6	21.4	20.5
5	P3U	2200	81.0	24.7	21.0	20.6	19.9
6	P3U	2300	81.0	23.4	20.5	20.1	19.6
7	P3U	2400	81.0	23.0	19.8	19.5	19.1
8	P3U	0100	81.0	22.8	19.3	19.0	18.5
9	P3U	0200	81.0	22.6	18.8	18.5	17.9
10	P3U	0300	82.0	21.7	18.2	18.0	17.7
11	P3U	0400	82.0	21.7	18.0	17.8	17.4
12	P3U	0500	83.5	20.6	17.2	17.1	16.7
13	P3U	0600	82.0	20.4	16.9	16.7	16.2
14	P3U	0700	83.0	20.9	17.6	17.6	17.3
15	P3U	0800	82.0	21.2	18.8	19.0	18.7
16	P3U	0900	79.0	26.3	26.9	29.5	29.1
17	P3U	1000	65.5	29.8	31.5	33.4	36.4
18	P3U	1100	60.6	30.7	33.4	33.7	36.4
19	P3U	1200	53.0	32.9	34.8	36.1	36.7
20	P3U	1300	42.0	34.5	37.7	39.0	43.4
21	P3U	1400	45.0	34.8	39.4	39.4	43.2
22	P3U	1500	41.5	35.4	40.6	42.5	44.3
23	P3U	1600	61.5	31.4	33.7	34.1	33.3
24	P3U	1700	67.5	29.7	30.2	30.1	29.6

Table S4. Thermographic data of *N. khasiana* unopen pitcher P4U.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3
1	P4U	1800	60.0	29.0	28.5	27.3	26.2
2	P4U	1900	63.0	28.2	25.4	24.5	23.6
3	P4U	2000	68.0	28.0	24.6	24.4	23.4
4	P4U	2100	70.0	27.5	22.8	22.3	21.1
5	P4U	2200	78.0	26.0	21.2	20.8	20.0
6	P4U	2300	80.0	25.2	21.1	20.6	19.8
7	P4U	2400	80.0	25.0	20.0	19.4	18.5
8	P4U	0100	80.0	23.5	18.9	18.6	17.9
9	P4U	0200	81.0	22.0	17.8	17.1	16.5
10	P4U	0300	80.0	21.5	16.7	16.3	15.7
11	P4U	0400	81.0	21.3	15.7	15.3	14.6
12	P4U	0500	80.0	21.2	15.6	15.4	14.9
13	P4U	0600	82.0	19.0	15.4	15.2	14.7
14	P4U	0700	79.0	21.0	15.2	14.9	14.4
15	P4U	0800	79.0	22.0	18.3	18.8	18.3
16	P4U	0900	73.0	26.0	23.4	23.3	22.1
17	P4U	1000	55.0	30.7	30.0	30.2	30.0
18	P4U	1100	50.0	32.5	33.6	34.2	35.1
19	P4U	1200	47.0	34.5	35.9	36.7	41.0
20	P4U	1300	46.0	35.0	37.4	35.1	34.1
21	P4U	1400	44.0	35.5	41.5	42.9	45.4
22	P4U	1500	44.0	34.2	40.1	40.6	42.8
23	P4U	1600	46.9	33.8	35.3	33.9	34.1
24	P4U	1700	59.4	30.7	29.9	29.0	28.6

Table S5. Thermographic data of *N. khasiana* unopen pitcher P5U.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3
1	P5U	1800	60.0	29.0	27.6	27.5	26.1
2	P5U	1900	63.0	28.2	25.2	25.1	23.3
3	P5U	2000	68.0	28.0	24.8	25.0	23.2
4	P5U	2100	70.0	27.5	22.9	22.8	21.0
5	P5U	2200	78.0	26.0	21.7	21.5	20.4
6	P5U	2300	80.0	25.2	21.2	21.1	19.6
7	P5U	2400	80.0	25.0	20.2	20.0	18.6
8	P5U	0100	80.0	23.5	19.4	19.2	17.9
9	P5U	0200	81.0	22.0	18.5	18.1	17.0
10	P5U	0300	80.0	21.5	17.4	17.3	16.2
11	P5U	0400	81.0	21.3	16.8	16.7	15.4
12	P5U	0500	80.0	21.2	16.4	16.1	15.0
13	P5U	0600	82.0	19.0	15.6	15.5	14.4
14	P5U	0700	79.0	21.0	15.9	15.7	14.9
15	P5U	0800	79.0	22.0	18.8	19.2	17.9
16	P5U	0900	73.0	26.0	24.0	25.0	21.7
17	P5U	1000	55.0	30.7	30.7	30.7	29.6
18	P5U	1100	50.0	32.5	34.1	34.7	39.9
19	P5U	1200	47.0	34.5	34.8	34.8	39.7
20	P5U	1300	46.0	35.0	37.2	37.8	43.1
21	P5U	1400	44.0	35.5	40.7	41.6	45.6
22	P5U	1500	44.0	34.2	40.5	41.1	42.7
23	P5U	1600	46.9	33.8	33.3	32.8	32.0
24	P5U	1700	59.4	30.7	30.0	29.6	28.6

Table S6. Thermographic data of *N. khasiana* unopen pitcher P6U.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3
1	P6U	1800	60.0	29.0	28.1	27.5	26.7
2	P6U	1900	63.0	28.2	25.4	24.9	24.3
3	P6U	2000	68.0	28.0	24.6	24.6	23.7
4	P6U	2100	70.0	27.5	23.1	22.4	21.6
5	P6U	2200	78.0	26.0	22.0	21.4	20.4
6	P6U	2300	80.0	25.2	20.9	20.2	19.4
7	P6U	2400	80.0	25.0	20.1	19.2	18.7
8	P6U	0100	80.0	23.5	19.3	18.7	18.1
9	P6U	0200	81.0	22.0	18.0	17.4	16.3
10	P6U	0300	80.0	21.5	17.1	16.5	16.2
11	P6U	0400	81.0	21.3	16.2	15.8	15.8
12	P6U	0500	80.0	21.2	16.1	15.7	15.5
13	P6U	0600	82.0	19.0	15.6	15.0	14.8
14	P6U	0700	79.0	21.0	15.8	15.4	15.2
15	P6U	0800	79.0	22.0	19.5	20.0	19.5
16	P6U	0900	73.0	26.0	25.7	26.7	25.9
17	P6U	1000	55.0	30.7	30.8	36.4	41.3
18	P6U	1100	50.0	32.5	37.3	33.1	31.6
19	P6U	1200	47.0	34.5	34.8	33.6	33.5
20	P6U	1300	46.0	35.0	38.5	35.4	36.7
21	P6U	1400	44.0	35.5	37.2	35.1	35.3
22	P6U	1500	44.0	34.2	36.9	35.4	35.7
23	P6U	1600	46.9	33.8	33.7	31.4	31.6
24	P6U	1700	59.4	30.7	30.2	28.9	29.1

Table S7. Thermographic data of *N. khasiana* open pitcher P1O.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3	sp4	sp5
1	P1O	1800	71.0	27.8	27.2	26.7	25.6	25.7	24.7
2	P1O	1900	77.0	26.1	23.7	23.4	22.8	22.5	22.4
3	P1O	2000	78.1	24.9	22.5	22.2	21.8	21.7	21.7
4	P1O	2100	77.3	25.2	21.6	21.4	21.1	21.0	20.9
5	P1O	2200	81.0	24.7	20.7	20.6	20.4	20.2	20.2
6	P1O	2300	81.0	23.4	20.0	19.7	19.5	19.5	19.3
7	P1O	2400	81.0	23.0	19.7	19.6	19.5	19.2	19.2
8	P1O	0100	81.0	22.8	19.1	19.0	18.8	18.6	18.5
9	P1O	0200	81.0	22.6	18.5	18.3	18.1	18.0	17.8
10	P1O	0300	82.0	21.7	18.3	18.0	17.8	17.7	17.4
11	P1O	0400	82.0	21.7	17.9	17.7	17.5	17.3	17.2
12	P1O	0500	83.5	20.6	17.5	17.3	17.0	16.9	16.9
13	P1O	0600	82.0	20.4	17.3	17.1	16.9	16.9	16.7
14	P1O	0700	83.0	20.9	17.9	17.9	17.7	17.5	17.4
15	P1O	0800	82.0	21.2	19.0	19.2	18.8	18.7	18.5
16	P1O	0900	79.0	26.3	24.3	29.5	26.1	23.2	23.7
17	P1O	1000	65.5	29.8	30.1	35.0	34.4	30.2	33.9
18	P1O	1100	60.6	30.7	33.2	37.2	36.6	33.7	36.2
19	P1O	1200	53.0	32.9	35.3	35.1	34.7	33.1	36.1
20	P1O	1300	42.0	34.5	36.4	37.0	36.1	36.6	38.2
21	P1O	1400	45.0	34.8	36.7	36.5	33.1	34.0	32.9
22	P1O	1500	41.5	35.4	35.7	33.4	31.7	32.8	32.5
23	P1O	1600	61.5	31.4	31.5	30.6	29.5	30.0	29.9
24	P1O	1700	67.5	29.7	29.1	28.5	27.5	27.7	27.0

Table S8. Thermographic data of *N. khasiana* open pitcher P2O.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3	sp4	sp5
1	P2O	1800	71.0	27.8	27.3	26.8	26.4	25.8	25.7
2	P2O	1900	77.0	26.1	24.1	23.7	23.2	22.7	21.7
3	P2O	2000	78.1	24.9	22.7	22.5	22.1	21.5	20.5
4	P2O	2100	77.3	25.2	21.4	21.3	20.9	20.6	19.8
5	P2O	2200	81.0	24.7	20.9	20.8	20.5	20.1	19.5
6	P2O	2300	81.0	23.4	20.5	20.7	20.4	20.1	19.7
7	P2O	2400	81.0	23.0	19.7	19.6	19.2	18.8	18.5
8	P2O	0100	81.0	22.8	19.1	19.0	18.7	18.3	18.0
9	P2O	0200	81.0	22.6	18.5	18.5	18.1	17.7	17.5
10	P2O	0300	82.0	21.7	18.3	18.2	17.8	17.8	17.1
11	P2O	0400	82.0	21.7	17.9	18.0	17.7	17.6	17.2
12	P2O	0500	83.5	20.6	17.3	17.2	16.8	16.7	16.1
13	P2O	0600	82.0	20.4	16.9	16.9	17.1	16.3	16.1
14	P2O	0700	83.0	20.9	17.6	17.8	17.6	17.4	16.9
15	P2O	0800	82.0	21.2	18.6	19.1	18.8	18.5	18.0
16	P2O	0900	79.0	26.3	23.6	24.4	24.1	23.3	22.6
17	P2O	1000	65.5	29.8	30.2	29.7	29.5	29.9	28.8
18	P2O	1100	60.6	30.7	32.0	32.0	32.0	32.6	30.0
19	P2O	1200	53.0	32.9	33.6	33.7	34.9	36.9	37.1
20	P2O	1300	42.0	34.5	36.3	35.4	36.1	39.5	39.2
21	P2O	1400	45.0	34.8	38.5	36.2	36.5	39.3	40.3
22	P2O	1500	41.5	35.4	36.7	37.9	35.6	34.8	35.6
23	P2O	1600	61.5	31.4	32.8	32.6	32.7	34.4	31.8
24	P2O	1700	67.5	29.7	29.6	29.1	29.2	29.1	28.2

Table S9. Thermographic data of *N. khasiana* open pitcher P3O.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3	sp4	sp5
1	P3O	1800	71.0	27.8	26.6	26.2	25.7	26.0	26.0
2	P3O	1900	77.0	26.1	23.3	23.1	22.7	22.9	22.8
3	P3O	2000	78.1	24.9	22.1	22.0	21.8	22.0	21.9
4	P3O	2100	77.3	25.2	20.9	20.8	20.7	20.9	20.8
5	P3O	2200	81.0	24.7	20.8	20.7	20.6	20.8	20.8
6	P3O	2300	81.0	23.4	20.1	20.0	19.8	20.0	19.9
7	P3O	2400	81.0	23.0	19.7	19.6	19.6	19.7	19.7
8	P3O	0100	81.0	22.8	19.1	19.0	19.0	19.0	19.0
9	P3O	0200	81.0	22.6	18.4	18.4	18.3	18.3	18.3
10	P3O	0300	82.0	21.7	18.1	17.9	17.9	17.9	18.0
11	P3O	0400	82.0	21.7	17.7	17.7	17.6	17.7	17.8
12	P3O	0500	83.5	20.6	17.1	16.9	16.9	17.0	16.9
13	P3O	0600	82.0	20.4	16.7	16.6	16.5	16.6	16.6
14	P3O	0700	83.0	20.9	17.4	17.5	17.4	17.5	17.5
15	P3O	0800	82.0	21.2	18.5	18.6	18.5	18.6	18.7
16	P3O	0900	79.0	26.3	23.0	23.0	22.7	23.0	23.1
17	P3O	1000	65.5	29.8	28.2	28.2	26.3	27.9	27.1
18	P3O	1100	60.6	30.7	30.9	30.7	29.6	30.9	32.2
19	P3O	1200	53.0	32.9	31.6	31.9	30.8	31.3	32.8
20	P3O	1300	42.0	34.5	33.2	32.9	31.9	32.3	32.9
21	P3O	1400	45.0	34.8	33.7	33.0	33.5	33.0	34.2
22	P3O	1500	41.5	35.4	33.5	33.2	32.6	32.4	33.1
23	P3O	1600	61.5	31.4	30.4	30.4	29.4	29.7	30.4
24	P3O	1700	67.5	29.7	29.3	29.0	28.0	28.4	28.6

Table S10. Thermographic data of *N. khasiana* open pitcher P4O.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3	sp4	sp5
1	P4O	1800	60.0	29.0	28.5	27.3	26.0	26.4	26.9
2	P4O	1900	63.0	28.2	25.4	24.6	24.2	24.2	24.1
3	P4O	2000	68.0	28.0	25.1	24.6	24.2	24.3	24.1
4	P4O	2100	70.0	27.5	23.3	22.5	22.2	22.6	22.1
5	P4O	2200	78.0	26.0	22.2	21.7	21.6	21.8	21.6
6	P4O	2300	80.0	25.2	21.4	20.9	20.7	21.0	20.8
7	P4O	2400	80.0	25.0	20.8	20.2	20.0	20.4	20.1
8	P4O	0100	80.0	23.5	19.7	19.2	18.9	19.4	19.2
9	P4O	0200	81.0	22.0	18.4	17.8	17.7	17.8	17.6
10	P4O	0300	80.0	21.5	17.6	17.2	17.0	17.3	17.1
11	P4O	0400	81.0	21.3	16.8	16.2	16.0	16.4	16.0
12	P4O	0500	80.0	21.2	16.3	15.9	15.8	15.9	15.8
13	P4O	0600	82.0	19.0	15.9	15.4	15.2	15.4	15.3
14	P4O	0700	79.0	21.0	15.9	15.5	15.5	15.5	15.5
15	P4O	0800	79.0	22.0	19.4	19.2	18.9	19.0	18.8
16	P4O	0900	73.0	26.0	24.4	24.1	22.8	22.7	22.8
17	P4O	1000	55.0	30.7	30.5	29.4	28.2	28.7	28.7
18	P4O	1100	50.0	32.5	35.1	34.1	34.9	36.9	44.3
19	P4O	1200	47.0	34.5	35.9	34.1	34.6	34.1	36.0
20	P4O	1300	46.0	35.0	36.9	34.6	33.7	34.4	35.3
21	P4O	1400	44.0	35.5	40.9	42.2	35.8	36.1	37.0
22	P4O	1500	44.0	34.2	38.2	40.7	40.7	35.1	42.1
23	P4O	1600	46.9	33.8	33.9	33.5	33.2	31.6	33.4
24	P4O	1700	59.4	30.7	30.0	29.1	28.1	28.1	28.6

Table S11. Thermographic data of *N. khasiana* open pitcher P5O.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3	sp4	sp5
1	P5O	1800	60.0	29.0	28.3	27.0	26.6	26.6	26.2
2	P5O	1900	63.0	28.2	25.4	24.8	24.8	24.4	23.5
3	P5O	2000	68.0	28.0	24.8	24.5	24.0	24.2	23.9
4	P5O	2100	70.0	27.5	23.0	22.2	21.9	22.1	22.1
5	P5O	2200	78.0	26.0	21.9	21.5	21.1	21.3	21.3
6	P5O	2300	80.0	25.2	21.1	20.6	20.5	20.5	20.6
7	P5O	2400	80.0	25.0	20.2	19.4	19.4	19.5	19.4
8	P5O	0100	80.0	23.5	19.4	18.7	18.7	18.7	18.7
9	P5O	0200	81.0	22.0	18.4	17.6	17.6	17.8	17.7
10	P5O	0300	80.0	21.5	17.0	16.5	16.6	16.5	16.7
11	P5O	0400	81.0	21.3	16.3	16.0	15.7	15.9	15.9
12	P5O	0500	80.0	21.2	16.0	15.4	15.4	15.4	15.5
13	P5O	0600	82.0	19.0	15.5	15.0	15.0	15.0	15.0
14	P5O	0700	79.0	21.0	15.9	15.5	15.5	15.5	15.5
15	P5O	0800	79.0	22.0	18.8	18.8	18.6	18.6	18.5
16	P5O	0900	73.0	26.0	24.1	23.0	22.2	22.3	22.1
17	P5O	1000	55.0	30.7	30.1	29.6	28.0	29.1	26.7
18	P5O	1100	50.0	32.5	33.1	32.7	31.8	34.2	41.9
19	P5O	1200	47.0	34.5	35.0	33.8	32.5	34.0	33.4
20	P5O	1300	46.0	35.0	38.3	37.4	36.1	41.5	36.1
21	P5O	1400	44.0	35.5	41.3	41.2	41.1	41.4	38.4
22	P5O	1500	44.0	34.2	41.1	41.9	40.9	40.3	38.4
23	P5O	1600	46.9	33.8	34.2	32.4	31.2	31.4	31.1
24	P5O	1700	59.4	30.7	30.0	29.0	28.5	28.6	28.6

Table S12. Thermographic data of *N. khasiana* open pitcher P6O.

Sl. No.	Pitcher number	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3	sp4	sp5
1	P6O	1800	60.0	29.0	28.0	27.2	26.6	26.4	25.6
2	P6O	1900	63.0	28.2	25.5	25.0	24.7	24.3	23.1
3	P6O	2000	68.0	28.0	25.4	25.0	24.6	24.5	23.2
4	P6O	2100	70.0	27.5	23.4	22.8	22.3	21.9	20.8
5	P6O	2200	78.0	26.0	22.0	21.8	21.6	21.3	20.5
6	P6O	2300	80.0	25.2	21.7	21.0	20.7	20.5	19.6
7	P6O	2400	80.0	25.0	20.4	19.8	19.5	19.5	18.7
8	P6O	0100	80.0	23.5	19.6	19.0	18.7	18.7	17.6
9	P6O	0200	81.0	22.0	18.6	17.8	17.4	17.2	16.5
10	P6O	0300	80.0	21.5	17.4	16.8	16.5	16.3	15.5
11	P6O	0400	81.0	21.3	16.7	16.3	16.0	16.1	14.7
12	P6O	0500	80.0	21.2	16.4	16.0	15.7	15.7	15.0
13	P6O	0600	82.0	19.0	16.2	15.8	15.5	15.4	14.6
14	P6O	0700	79.0	21.0	16.5	16.0	15.4	15.2	14.7
15	P6O	0800	79.0	22.0	19.8	19.6	19.3	18.8	17.8
16	P6O	0900	73.0	26.0	25.5	25.7	25.1	22.5	21.5
17	P6O	1000	55.0	30.7	30.6	30.1	30.2	29.9	28.7
18	P6O	1100	50.0	32.5	33.8	31.8	32.5	32.2	31.6
19	P6O	1200	47.0	34.5	35.6	33.7	34.0	36.8	35.0
20	P6O	1300	46.0	35.0	36.4	35.3	34.5	37.3	39.0
21	P6O	1400	44.0	35.5	38.2	38.2	36.0	38.1	42.0
22	P6O	1500	44.0	34.2	37.5	39.2	37.3	37.0	40.5
23	P6O	1600	46.9	33.8	32.7	31.2	31.1	31.0	30.7
24	P6O	1700	59.4	30.7	30.1	29.2	28.4	28.4	28.7

Fig. S1. Thermographic profiles of *Nepenthes* hybrids: (A) hybrid 1 unopen pitcher; (B) hybrid 2 unopen pitcher; (C) hybrid 1 open pitcher; (D) hybrid 2 open pitcher; (E) hybrid 3 open pitcher; (F) hybrid 4 open pitcher.

Sl. No./ spot	p.m.					a.m.									
	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9
P1U															
sp1															
sp2															
sp3															
P2U															
sp1															
sp2															
sp3															
P3U															
sp1															
sp2															
sp3															
P4U															
sp1															
sp2															
sp3															
P5U															
sp1															
sp2															
sp3															
P6U															
sp1															
sp2															
sp3															

Fig. S2. Nighttime duration (black shaded) in unopen *N. khasiana* pitcher spots (P1U to P6U) at > 3 degrees below the atmospheric temperature.

Sl. No./ spot	p.m.						a.m.										
	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10
P1O																	
sp1																	
sp2																	
sp3																	
sp4																	
sp5																	
P2O																	
sp1																	
sp2																	
sp3																	
sp4																	
sp5																	
P3O																	
sp1																	
sp2																	
sp3																	
sp4																	
sp5																	
P4O																	
sp1																	
sp2																	
sp3																	
sp4																	
sp5																	
P5O																	
sp1																	
sp2																	
sp3																	
sp4																	
sp5																	
P6O																	
sp1																	
sp2																	
sp3																	
sp4																	
sp5																	

Fig. S3. Nighttime duration (black shaded) in *N. khasiana* open pitcher spots (P1O to P6O) at > 3 degrees below the atmospheric temperature.

Table S13. Thermographic data of *Victoria amazonica* (January 2019).

Sl. No.	Species	Time	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3
1	*V. amazonica*	16.00	61.5	31.4	-	28.4	28.2
2	*V. amazonica*	17.00	67.5	29.7	-	29.3	26.7
3	*V. amazonica*	18.00	75.0	27.0	-	30.1	25.1
4	*V. amazonica*	19.00	80.0	25.8	28.6	29.6	25.2
5	*V. amazonica*	20.00	82.0	25.7	25.3	28.0	23.9
6	*V. amazonica*	21.00	81.0	25.2	23.4	27.9	22.9
7	*V. amazonica*	22.00	82.4	24.9	23.1	28.0	22.3
8	*V. amazonica*	23.00	82.2	24.3	23.2	27.0	21.8
9	*V. amazonica*	24.00	82.8	23.7	22.0	26.6	21.3
10	*V. amazonica*	01.00	80.8	23.8	21.7	25.5	20.8
11	*V. amazonica*	02.00	83.3	22.6	21.3	25.2	20.3
12	*V. amazonica*	03.00	82.5	22.6	21.0	24.6	19.6
13	*V. amazonica*	04.00	83.0	21.9	20.7	24.2	19.1
14	*V. amazonica*	05.00	81.6	22.0	19.6	23.6	19.1
15	*V. amazonica*	06.00	83.5	21.2	19.9	23.8	18.4
16	*V. amazonica*	07.00	85.3	20.5	20.1	23.6	19.4
17	*V. amazonica*	08.00	80.0	23.8	21.6	26.4	22.7
18	*V. amazonica*	09.00	78.0	26.8	27.1	32.1	31.3
19	*V. amazonica*	10.00	83.0	26.0	29.5	34.0	30.1

Table S14. Thermographic data of *V. amazonica* (January 2022).

Sl. No.	Species	Time	Humidity (%)	Temperature (^{o}C)	sp1	sp2	sp3
1	*V. amazonica*	16.00	65.0	33.0	-	28.6	29.5
2	*V. amazonica*	17.00	85.0	28.8	-	28.6	26.0
3	*V. amazonica*	18.00	84.0	27.2	-	28.9	25.4
4	*V. amazonica*	19.00	84.0	27.0	25.0	29.0	24.6
5	*V. amazonica*	20.00	83.5	26.7	25.1	27.7	24.2
6	*V. amazonica*	21.00	84.3	25.9	25.0	28.2	23.2
7	*V. amazonica*	22.00	83.0	26.0	23.6	27.3	22.8
8	*V. amazonica*	23.00	83.6	26.0	23.4	26.8	22.2
9	*V. amazonica*	24.00	83.7	25.7	23.3	26.1	22.3
10	*V. amazonica*	01.00	83.8	25.4	22.5	25.5	22.0
11	*V. amazonica*	02.00	82.4	25.4	22.6	24.0	21.2
12	*V. amazonica*	03.00	83.7	24.4	22.3	23.7	20.9
13	*V. amazonica*	04.00	82.0	24.0	21.6	23.8	20.3
14	*V. amazonica*	05.00	82.3	23.4	21.5	23.8	20.0
15	*V. amazonica*	06.00	83.0	23.0	21.3	22.7	20.1
16	*V. amazonica*	07.00	83.2	23.6	21.7	23.8	20.8
17	*V. amazonica*	08.00	84.0	24.3	23.3	26.3	22.9
18	*V. amazonica*	09.00	79.5	28.5	24.5	29.3	25.7
19	*V. amazonica*	10.00	79.0	28.9	26.4	29.1	26.3
20	*V. amazonica*	11.00	72.0	31.7	28.4	30.1	29.2
21	*V. amazonica*	12.00	64.1	31.8	29.1	31.0	30.8
22	*V. amazonica*	13.00	57.0	36.6	30.7	31.3	33.7
23	*V. amazonica*	14.00	64.3	31.5	29.4	30.5	30.4
24	*V. amazonica*	15.00	68.0	30.3	27.9	27.7	28.3
25	*V. amazonica*	16.00	76.0	28.5	28.1	28.8	27.8
26	*V. amazonica*	17.00	74.5	30.3	27.7	28.3	26.9
27	*V. amazonica*	18.00	76.0	28.5	26.2	27.0	24.9
28	*V. amazonica*	19.00	81.2	27.0	25.6	28.8	24.2
29	*V. amazonica*	20.00	80.0	27.1	25.9	31.0	23.5
30	*V. amazonica*	21.00	83.1	26.2	25.9	30.0	25.2
31	*V. amazonica*	22.00	81.8	26.0	25.7	28.8	23.5
32	*V. amazonica*	23.00	82.8	26.1	25.2	27.9	23.5
33	*V. amazonica*	24.00	81.5	26.0	24.1	26.8	22.8
34	*V. amazonica*	01.00	84.1	25.0	24.1	26.4	23.0
35	*V. amazonica*	02.00	83.6	24.9	24.0	26.0	22.1
36	*V. amazonica*	03.00	83.0	26.0	24.3	25.4	22.7
37	*V. amazonica*	04.00	83.8	24.6	23.7	25.3	22.0

38	*V. amazonica*	05.00	83.0	24.3	23.6	24.7	22.7
39	*V. amazonica*	06.00	82.5	23.3	23.5	23.5	21.4
40	*V. amazonica*	07.00	84.9	23.7	23.6	23.8	22.6
41	*V. amazonica*	08.00	82.7	26.2	24.9	25.7	24.5
42	*V. amazonica*	09.00	78.0	29.1	27.8	30.8	29.3
43	*V. amazonica*	10.00	65.5	31.6	29.8	34.5	30.1
44	*V. amazonica*	11.00	75.5	29.4	28.3	30.7	30.1
45	*V. amazonica*	12.00	73.0	30.6	28.5	32.2	31.8
46	*V. amazonica*	13.00	65.5	30.6	30.3	33.7	32.7
47	*V. amazonica*	14.00	65.5	34.2	38.0	37.1	40.2
48	*V. amazonica*	15.00	75.7	31.5	28.2	30.9	30.5

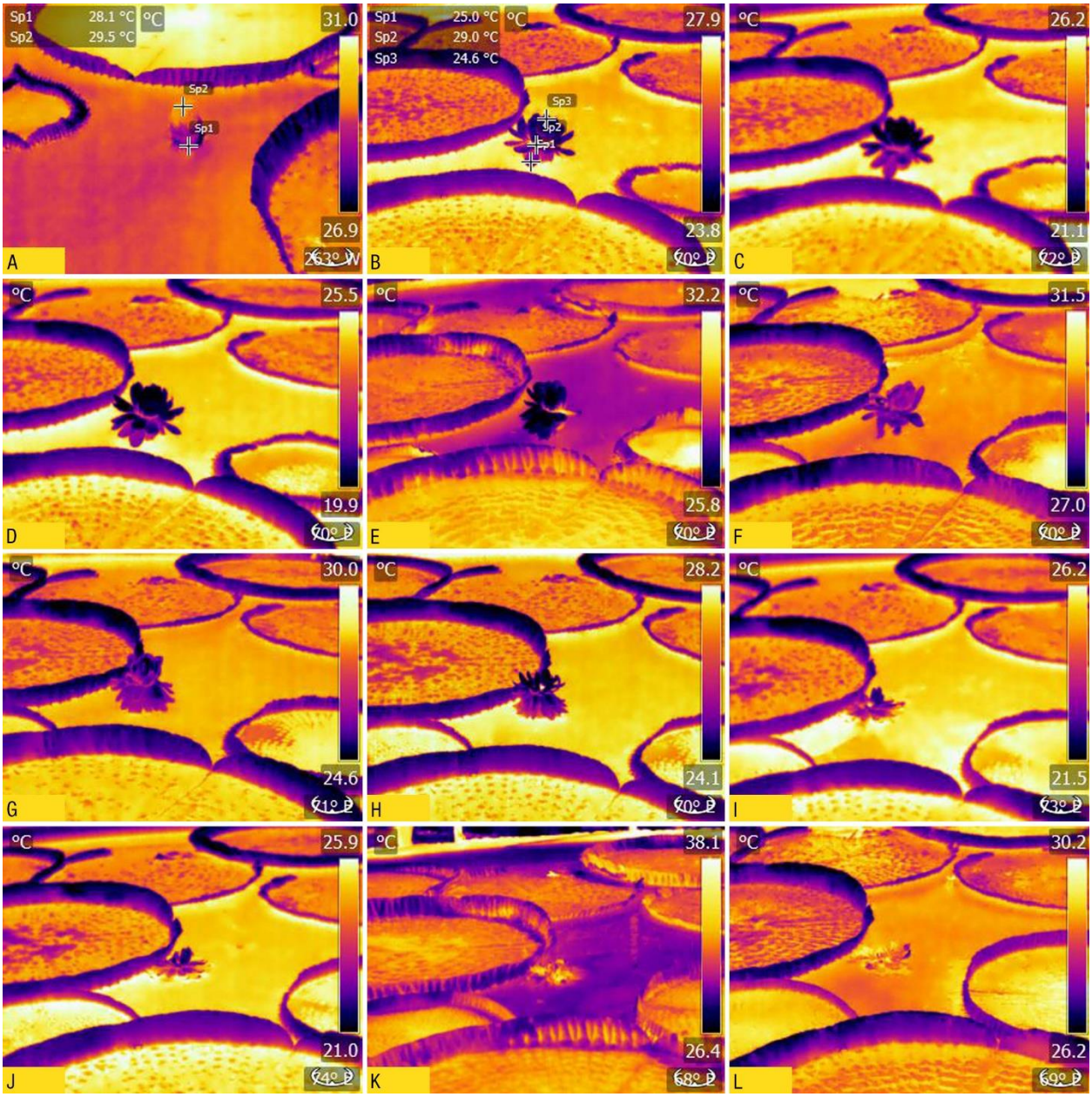

Fig. S4. Thermographic profiles of *V. amazonica* (A) flower bud above the level of water (1600); (B) flower bloomed, three clear spots (sp1, sp2, sp3) visible (1900); (C & D) flower showing three spots but intensity of sp2 decreased (0200 & 0600); (E) flower is slightly pink and started to fold (1000); (F) flower in completely pink, partially opened, sp2 is visible but with low intensity (1600); (G) flower fully opened and sp2 is of low intensity (1800); (H) flower top is more opened and a hot central portion is visible (1900); (I) flower is partially floating in water, central region is visible (0200); (J) flower is partially floating, central region visible with low intensity (0600); (K) flower floats above

water not spot differentiation visible (1000); (L) flower almost immersed in water with some portions floating above, nearly dead state (1600).

Table S15. Thermographic data of *Nelumbo nucifera* (March 2021).

Sl. No.	Species	Time (h)	Humidity (%)	Temperature (ºC)	sp1	sp2	sp3
1	*N. nucifera*	1800	60.0	29.0	27.3	25.2	30.3
2	*N. nucifera*	1900	63.0	28.2	24.6	26.7	29.2
3	*N. nucifera*	2000	68.0	28.0	24.2	26.3	30.2
4	*N. nucifera*	2100	70.0	27.5	22.8	25.7	30.6
5	*N. nucifera*	2200	78.0	26.0	22.0	24.7	29.9
6	*N. nucifera*	2300	80.0	25.2	21.3	24.5	28.6
7	*N. nucifera*	2400	80.0	25.0	20.5	23.0	27.3
8	*N. nucifera*	0100	80.0	23.5	19.3	21.4	25.4
9	*N. nucifera*	0200	81.0	22.0	18.2	20.2	24.4
10	*N. nucifera*	0300	80.0	21.5	17.1	18.3	23.0
11	*N. nucifera*	0400	81.0	21.3	16.7	18.2	21.4
12	*N. nucifera*	0500	80.0	21.2	16.1	18.3	20.9
13	*N. nucifera*	0600	82.0	19.0	15.7	16.6	20.4
14	*N. nucifera*	0700	79.0	21.0	16.7	17.4	18.9
15	*N. nucifera*	0800	79.0	22.0	22.6	23.1	29.7
16	*N. nucifera*	0900	73.0	26.0	24.5	24.8	30.1
17	*N. nucifera*	1000	55.0	30.7	27.7	28.8	33.3
18	*N. nucifera*	1100	50.0	32.5	35.2	37.4	39.1
19	*N. nucifera*	1200	47.0	34.5	34.9	38.3	38.6
20	*N. nucifera*	1300	46.0	35.0	36.9	42.7	39.2
21	*N. nucifera*	1400	44.0	35.5	38.8	39.1	37.6
22	*N. nucifera*	1500	44.0	34.2	35.9	36.4	34.8
23	*N. nucifera*	1600	46.9	33.8	31.4	32.1	32.7
24	*N. nucifera*	1700	59.4	30.7	27.9	27.9	29.1

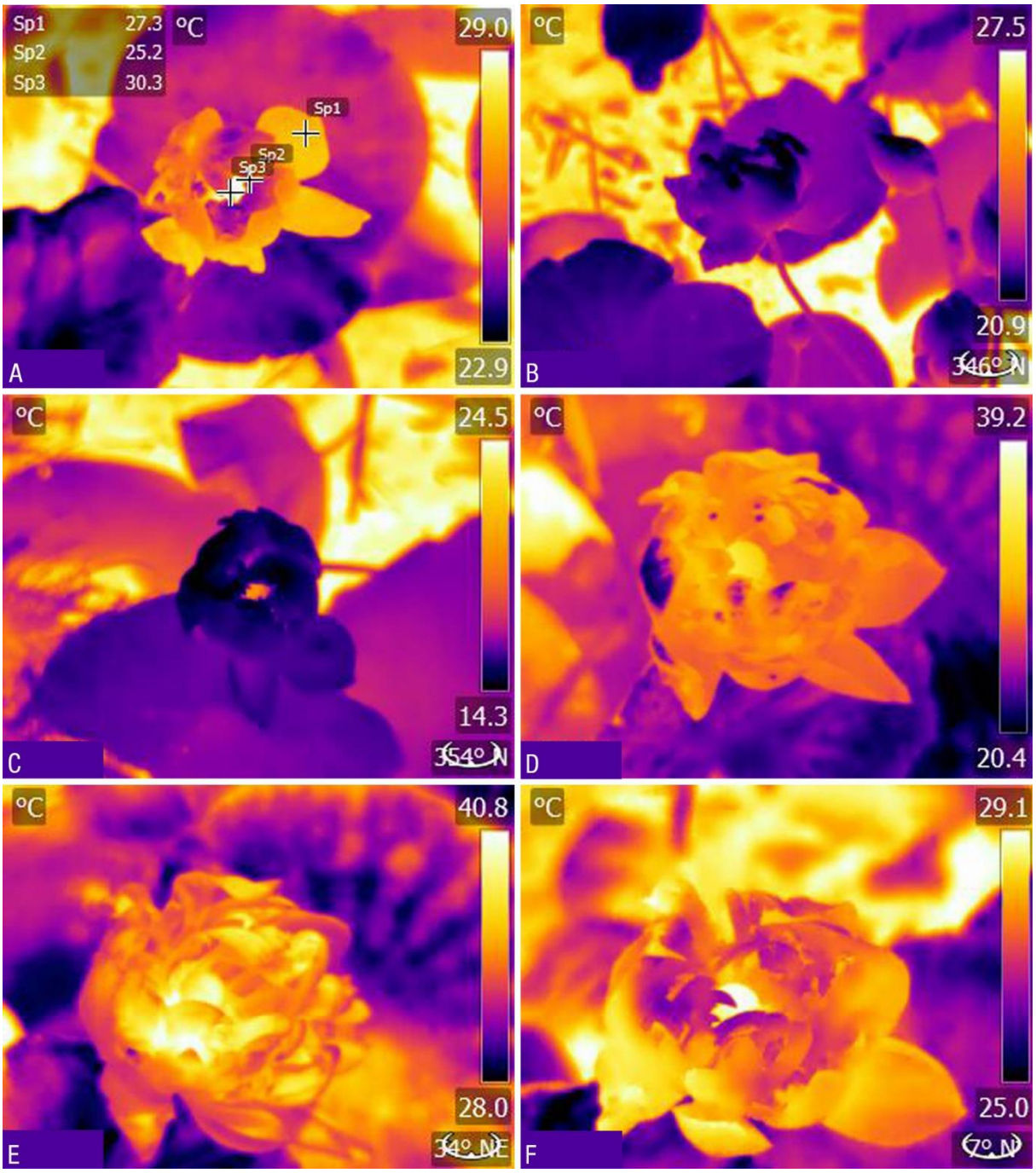

Fig. S5. Thermographic profiles of *N. nucifera* (A) fully bloomed flower in open pond, three regions clearly spotted (1800); (B) flower partially closed, three regions not clearly visible (2200); (C) flower partially opened, regions begins to appear (0600); (D) flower completely opened, all regions clearly spottable (1000); (E) flower, totally opened and is moisture free (1100); (F) flower began to close, all regions visible (1800).

Table S16. Thermographic data of *Spathiphyllum cannifolium* (January 2019).

Sl. No.	Species	Time (h)	Humidity (%)	Temperature (^{o}C)	sp1	sp2	sp3
1	*S. cannifolium*	1800	71.0	27.8	25.6	25.5	25.5
2	*S. cannifolium*	1900	77.0	26.1	23.1	23.2	23.0
3	*S. cannifolium*	2000	78.1	24.9	22.3	22.2	22.4
4	*S. cannifolium*	2100	77.3	25.2	20.9	21.1	21.0
5	*S. cannifolium*	2200	81.0	24.7	20.7	20.9	20.8
6	*S. cannifolium*	2300	81.0	23.4	20.1	20.2	20.3
7	*S. cannifolium*	2400	81.0	23.0	19.6	19.6	19.7
8	*S. cannifolium*	0100	81.0	22.8	19.0	19.1	19.1
9	*S. cannifolium*	0200	81.0	22.6	18.4	18.6	18.5
10	*S. cannifolium*	0300	82.0	21.7	18.1	18.2	18.2
11	*S. cannifolium*	0400	83.0	21.3	17.9	18.0	18.1
12	*S. cannifolium*	0500	83.5	20.6	17.1	17.1	17.2
13	*S. cannifolium*	0600	82.0	20.4	16.8	16.8	16.8
14	*S. cannifolium*	0700	83.0	20.9	17.6	17.7	17.8
15	*S. cannifolium*	0800	82.0	21.2	18.6	18.6	18.7
16	*S. cannifolium*	0900	79.0	26.3	22.7	22.7	23.0
17	*S. cannifolium*	1000	65.5	29.8	28.1	27.7	28.1
18	*S. cannifolium*	1100	60.6	30.7	29.6	29.2	29.2
19	*S. cannifolium*	1200	53.0	32.9	31.4	30.6	31.0
20	*S. cannifolium*	1300	42.5	34.5	33.5	31.9	32.8
21	*S. cannifolium*	1400	45.0	34.8	33.6	32.2	32.9
22	*S. cannifolium*	1500	41.5	35.4	33.2	32.3	32.5
23	*S. cannifolium*	1600	61.5	31.4	31.7	30.5	30.5
24	*S. cannifolium*	1700	67.5	29.7	28.6	28.5	28.5

Table S17. Thermographic data of *S. wallisii* (January 2019).

Sl. No.	Species	Time (h)	Humidity (%)	Temperature (^{o}C)	sp1	sp2	sp3
1	*S. wallisii*	1800	71.0	27.8	25.8	26.7	26.6
2	*S. wallisii*	1900	77.0	26.1	23.5	24.2	24.0
3	*S. wallisii*	2000	78.1	24.9	22.0	22.7	22.6
4	*S. wallisii*	2100	77.3	25.2	21.5	21.8	21.8
5	*S. wallisii*	2200	81.0	24.7	20.8	21.2	21.2
6	*S. wallisii*	2300	81.0	23.4	20.3	20.6	20.7
7	*S. wallisii*	2400	81.0	23.0	19.6	19.9	19.9
8	*S. wallisii*	0100	81.0	22.8	19.0	19.4	19.4
9	*S. wallisii*	0200	81.0	22.6	18.4	18.8	18.8
10	*S. wallisii*	0300	82.0	21.7	18.1	18.4	18.4
11	*S. wallisii*	0400	83.0	21.3	17.9	18.2	18.1
12	*S. wallisii*	0500	83.5	20.6	16.9	17.2	17.2
13	*S. wallisii*	0600	82.0	20.4	16.8	17.1	17.2
14	*S. wallisii*	0700	83.0	20.9	17.5	17.7	17.8
15	*S. wallisii*	0800	82.0	21.2	18.8	19.0	19.0
16	*S. wallisii*	0900	79.0	26.3	22.4	23.0	23.0
17	*S. wallisii*	1000	65.5	29.8	28.4	28.3	28.4
18	*S. wallisii*	1100	60.6	30.7	31.0	30.7	30.7
19	*S. wallisii*	1200	53.0	32.9	37.3	34.2	36.2
20	*S. wallisii*	1300	42.5	34.5	37.8	35.0	37.0
21	*S. wallisii*	1400	45.0	34.8	38.3	35.7	37.8
22	*S. wallisii*	1500	41.5	35.4	36.9	34.8	36.3
23	*S. wallisii*	1600	61.5	31.4	30.6	30.7	30.7
24	*S. wallisii*	1700	67.5	29.7	29.3	29.5	29.3

Table S18. Thermographic data of *Aglaonema commutatum* (March 2021)

Sl. No.	Species	Time (h)	Humidity (%)	Temperature (^{o}C)	sp1	sp2	sp3
1	*A. commutatum*	1800	60.0	29.0	26.5	25.6	25.4
2	*A. commutatum*	1900	63.0	28.2	25.2	23.9	23.8
3	*A. commutatum*	2000	68.0	28.0	24.1	23.9	23.8
4	*A. commutatum*	2100	70.0	27.5	22.6	22.8	22.6
5	*A. commutatum*	2200	78.0	26.0	21.2	21.3	21.3
6	*A. commutatum*	2300	80.0	25.2	20.8	20.9	20.8
7	*A. commutatum*	2400	80.0	25.0	20.1	20.1	20.1
8	*A. commutatum*	0100	80.0	23.5	19.2	19.3	19.3
9	*A. commutatum*	0200	81.0	22.0	18.1	18.2	18.2
10	*A. commutatum*	0300	80.0	21.5	17.0	17.0	17.0
11	*A. commutatum*	0400	81.0	21.3	16.3	16.4	16.4
12	*A. commutatum*	0500	80.0	21.2	15.8	16.1	16.0
13	*A. commutatum*	0600	82.0	19.0	14.9	15.1	15.0
14	*A. commutatum*	0700	79.0	21.0	15.8	15.9	15.8
15	*A. commutatum*	0800	79.0	22.0	18.9	18.8	18.8
16	*A. commutatum*	0900	73.0	26.0	22.8	22.3	22.2
17	*A. commutatum*	1000	55.0	30.7	31.5	30.4	29.7
18	*A. commutatum*	1100	50.0	32.5	32.7	31.7	31.2
19	*A. commutatum*	1200	47.0	34.5	34.0	32.0	32.9
20	*A. commutatum*	1300	46.0	35.0	34.2	32.9	32.9
21	*A. commutatum*	1400	44.0	35.5	35.2	32.2	32.9
22	*A. commutatum*	1500	44.0	34.2	34.0	31.5	31.6
23	*A. commutatum*	1600	46.9	33.8	31.4	28.4	28.5
24	*A. commutatum*	1700	59.4	30.7	28.2	26.8	26.8

Table S19. Thermographic data of rose hybrid 1 (March 2021).

Sl. No.	Species	Time (h)	Humidity (%)	Temperature (^{o}C)	sp1	sp2
1	Rose hybrid 1	1800	60.0	29.0	26.3	25.2
2	Rose hybrid 1	1900	63.0	28.2	25.5	24.8
3	Rose hybrid 1	2000	68.0	28.0	23.8	23.6
4	Rose hybrid 1	2100	70.0	27.5	23.3	22.7
5	Rose hybrid 1	2200	78.0	26.0	21.6	21.5
6	Rose hybrid 1	2300	80.0	25.2	21.8	21.3
7	Rose hybrid 1	2400	80.0	25.0	20.3	20.1
8	Rose hybrid 1	0100	80.0	23.5	19.6	19.4
9	Rose hybrid 1	0200	81.0	22.0	18.9	18.5
10	Rose hybrid 1	0300	80.0	21.5	17.4	16.9
11	Rose hybrid 1	0400	81.0	21.3	16.8	16.2
12	Rose hybrid 1	0500	80.0	21.2	16.5	15.9
13	Rose hybrid 1	0600	82.0	19.0	15.7	15.4
14	Rose hybrid 1	0700	79.0	21.0	16.2	15.8
15	Rose hybrid 1	0800	79.0	22.0	18.9	18.9
16	Rose hybrid 1	0900	73.0	26.0	23.0	21.8
17	Rose hybrid 1	1000	55.0	30.7	31.7	32.0
18	Rose hybrid 1	1100	50.0	32.5	33.4	34.7
19	Rose hybrid 1	1200	47.0	34.5	34.7	36.4
20	Rose hybrid 1	1300	46.0	35.0	34.7	37.2
21	Rose hybrid 1	1400	44.0	35.5	37.2	36.1
22	Rose hybrid 1	1500	44.0	34.2	34.2	32.3
23	Rose hybrid 1	1600	46.9	33.8	32.0	31.4
24	Rose hybrid 1	1700	59.4	30.7	27.7	27.2

Table S20. Thermographic data of rose hybrid 2 (January 2019).

Sl. No.	Species	Time (h)	Humidity (%)	Temperature (^{o}C)	sp1	sp2
1	Rose hybrid 2	1800	71.0	27.8	25.5	25.1
2	Rose hybrid 2	1900	77.0	26.1	23.9	23.6
3	Rose hybrid 2	2000	78.1	24.9	22.3	22.3
4	Rose hybrid 2	2100	77.3	25.2	21.1	21.3
5	Rose hybrid 2	2200	81.0	24.7	21.1	21.2
6	Rose hybrid 2	2300	81.0	23.4	20.2	20.3
7	Rose hybrid 2	2400	81.0	23.0	19.8	19.9
8	Rose hybrid 2	0100	81.0	22.8	19.2	19.2
9	Rose hybrid 2	0200	81.0	22.6	18.6	18.5
10	Rose hybrid 2	0300	82.0	21.7	18.5	18.5
11	Rose hybrid 2	0400	83.0	21.3	17.9	17.9
12	Rose hybrid 2	0500	83.5	20.6	17.2	17.3
13	Rose hybrid 2	0600	82.0	20.4	17.0	17.0
14	Rose hybrid 2	0700	83.0	20.9	17.9	18.0
15	Rose hybrid 2	0800	82.0	21.2	19.2	19.1
16	Rose hybrid 2	0900	79.0	26.3	23.6	24.6
17	Rose hybrid 2	1000	65.5	29.8	29.0	28.7
18	Rose hybrid 2	1100	60.6	30.7	31.8	30.1
19	Rose hybrid 2	1200	53.0	32.9	33.4	30.5
20	Rose hybrid 2	1300	42.5	34.5	34.0	32.0
21	Rose hybrid 2	1400	45.0	34.8	33.9	30.4
22	Rose hybrid 2	1500	41.5	35.4	33.8	30.2
23	Rose hybrid 2	1600	61.5	31.4	30.9	28.7
24	Rose hybrid 2	1700	67.5	29.7	29.1	27.2

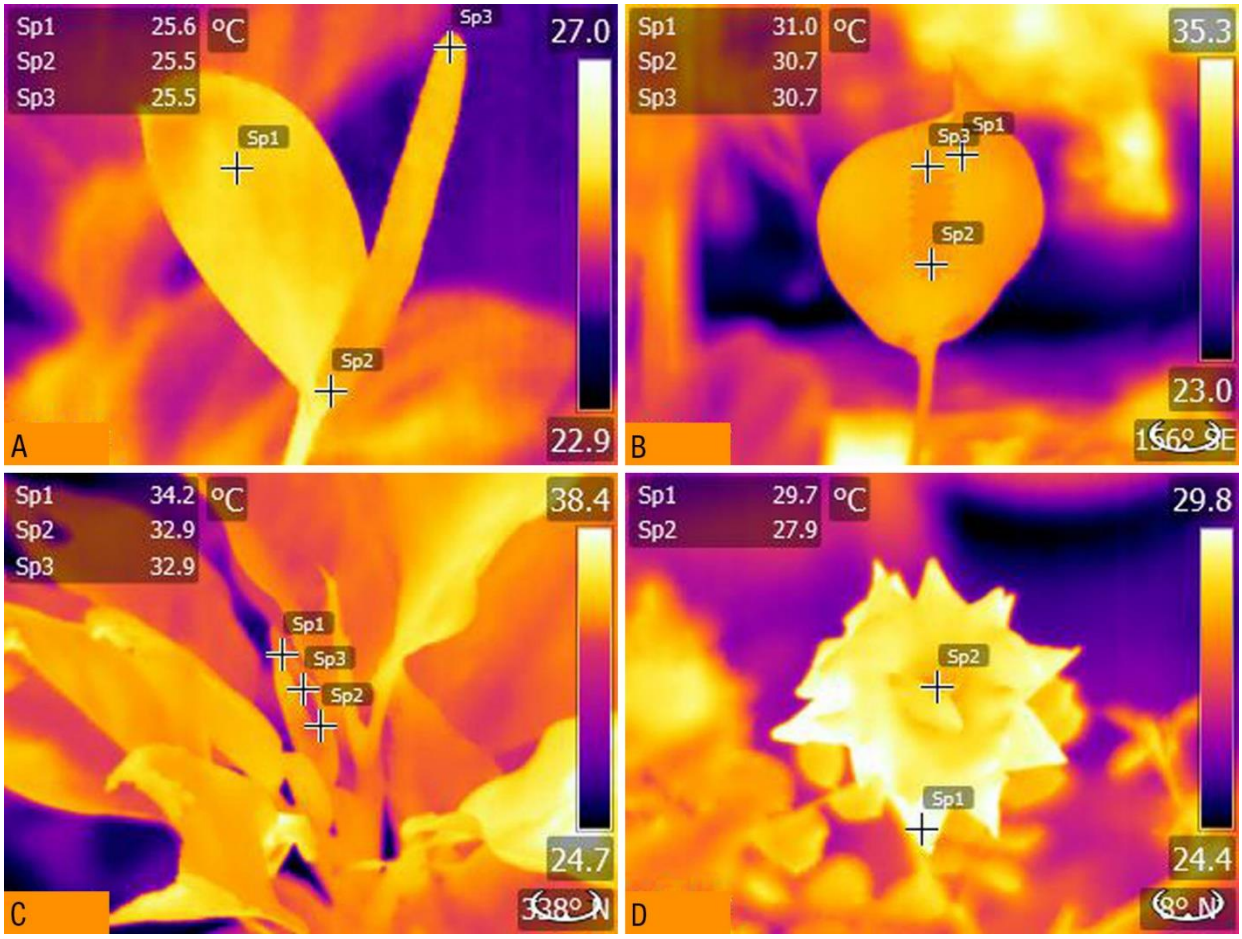

Fig. S6. Thermographic profiles of non-thermogenic flowers (A) *S. cannifolium*; (B) *S. wallisii*; (C) *A. commutatum* and (D) rose hybrid 1.

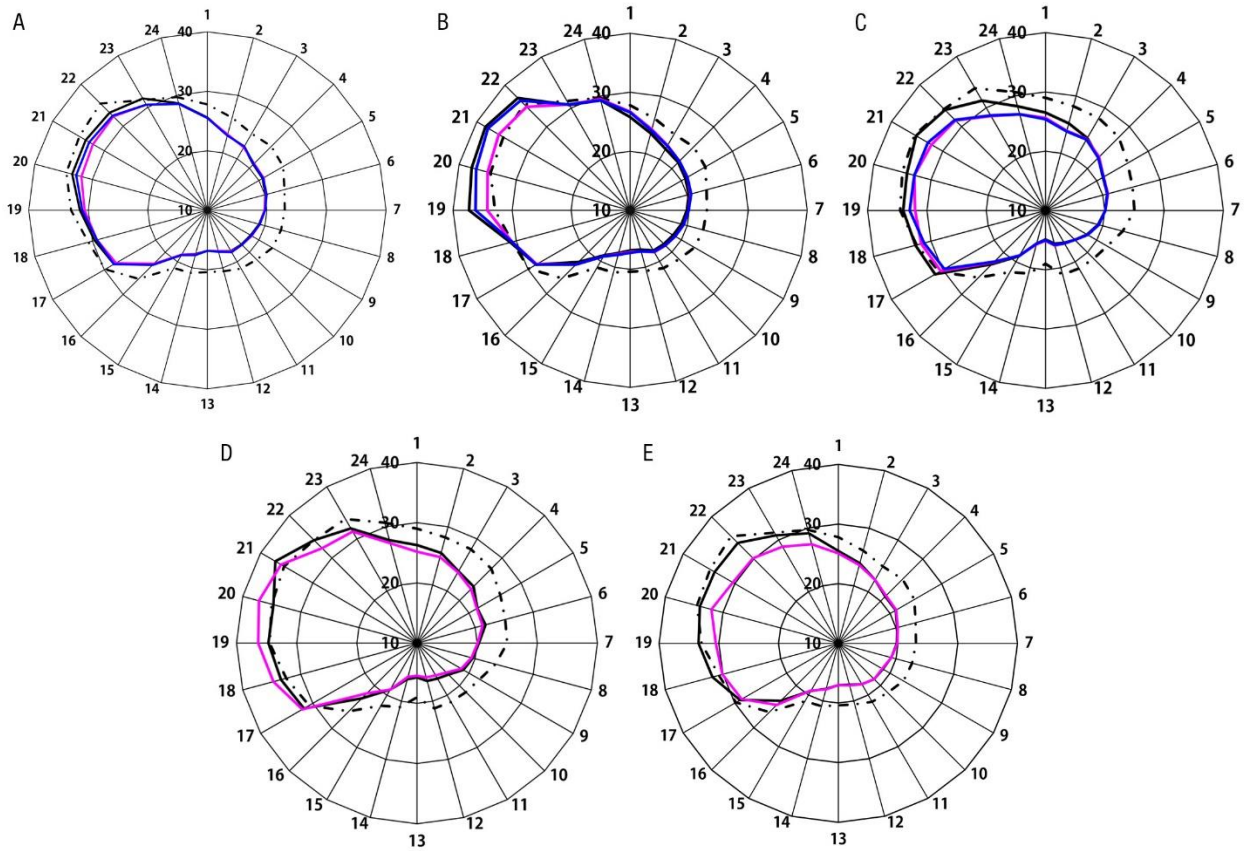

Fig. S7. Thermographic patterns of control flowers (A) *S. cannifolium*, (B) *S. wallisii*, (C) *A. commutatum*, and (D, E) two rose hybrid(s) in field conditions; (dotted line: ambient atm. temp., black: sp1, pink: sp2; blue: sp3; X-axis (circular) (A-E): 1-24 are 24 h time at 1 h measurement intervals starting 6 pm to 5 pm, next day (Tables S16-S20); Y-axis: temperature in degrees).