

1 **Differences in the reproductive behaviour and larval development**
2 **of two *Canthon rutilans* subspecies reinforce their thermal**
3 **regional segregation**

4
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17 **Short title:** *Thermal differences in subspecies behaviour*

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1 **Abstract**

2 Two dung beetle subspecies of *Canthon rutilans* Castelnau (Coleoptera: Scarabaeidae,
3 Scarabaeinae), *C. rutilans rutilans* and *C. rutilans cyanescens*, inhabit different environmental
4 temperature conditions in southern Brazil. We developed a laboratory breeding experiment
5 with 60 pairs of individuals of the two subspecies at five temperature conditions (from 15 to
6 35 °C) to compare the influence of temperature on the behaviour of adults and the
7 development of larvae. The behavioural patterns of the adults in both subspecies differ
8 according to temperature. The size of food balls was smaller and lighter in *C. rutilans*
9 *cyanescens*. Although temperature did not influence the feeding behaviour (measured as the
10 number of food balls made), reproductive behaviour (measured as the number of brood balls
11 made by pair) was significantly lower at 15 °C for both subspecies. Besides that, brood balls
12 from *C. rutilans rutilans* were bigger and heavier at lower temperatures. The number of
13 offspring and the time of emergence depend on temperature too. However, the weight of the
14 offspring and the longevity of adults depend on the subspecies and temperature treatment.
15 These results demonstrate that both subspecies have different thermal adaptations, where *C.*
16 *rutilans rutilans* has reproductive behaviour adapted to living under colder and broader
17 conditions than *C. rutilans cyanescens*.

18

19 **Abbreviated abstract (2-3 sentences, max. 80 words)**

20 Two spatially segregated dung beetle subspecies, *Canthon rutilans rutilans* and *C. rutilans*
21 *cyanescens* (Coleoptera: Scarabaeidae), were tested under various experimental thermal
22 conditions. Reproductive behaviour, larval development, and longevity of adults differed
23 depending on the temperature, where *C. rutilans rutilans* has reproductive behaviour adapted
24 to living under colder and broader conditions than *C. rutilans cyanescens*. These behavioural
25 and developmental adaptations support but not confirm that these two taxa can be considered
26 different species.

27

28

1 **Introduction**

2 Insects are exposed to climatic variation in temperature, humidity, atmospheric pressure,
3 wind, and precipitation, that may involve changes in their productivity, competition, and
4 predation (Rahbek, 1995; Lieberman et al., 1996; Lomolino, 2001). Temperature conditions
5 during development can affect the adults of many ectotherms (Atkinson, 1994; Atkinson &
6 Sibly, 1997). Insects have response mechanisms at non-freezing low temperatures (Chown &
7 Nicholson, 2004). The endothermic capacity for flight in some insects modifies their
8 geographical distribution, such as habitat selection (Blouin-Demers & Weatherhead, 2002;
9 Chown & Nicholson, 2004). Depending on their physiological limits, an insect species has its
10 own thermal niche, which limits its actual and potential range and which can be used to
11 explain species distribution (Verdú et al., 2006).

12 Dung beetle species (Coleoptera: Scarabaeidae, Scarabaeinae) are preferentially diversified
13 in tropical regions (Halffter, 1991) as temperature is a limiting factor for the development of
14 dung beetles inhabiting cold-temperate areas (Hanski, 1991). Similarly, the richness of
15 species decreases with elevation (Escobar et al., 2007; da Silva et al., 2018). At higher
16 elevations, the annual and daily thermal ranges are greater and dung beetle species have a
17 larger range of thermal tolerance than species at lower elevations (Gaston & Chown, 1999).
18 Species with capacity to regulate their body temperature have a higher resistance to low
19 temperatures (Verdú et al., 2006). Other adaptations to low temperatures include the use of
20 alternative trophic resources; e.g., the dung beetle *Thorectes lusitanicus* (Jekel) can also eat
21 acorns (*Quercus* spp.), making it more resistant to low temperatures (Pérez-Ramos et al.,
22 2007; Verdú et al., 2010). However, the dung beetle's most common trophic resource is
23 vertebrate faeces, which is used by both juveniles and adults (Halffter & Edmonds, 1982).

24 Recently, it was proposed that two subspecies of the Neotropical species *Canthon*
25 (*Francmonrosia*) *rutilans* Castelnau – the red *Canthon rutilans rutilans* Castelnau and the
26 blue *Canthon rutilans cyanescens* Harold (Figure 1) – may be two species, considering their
27 differing thermal preferences found in a regional study (Hensen et al., 2018). In southern
28 Brazil, these subspecies do not seem to co-occur: *C. rutilans rutilans* occurs in cool and
29 subtropical montane regions at more than 1 000 m elevation and adults are active at mean
30 environmental temperatures of 14.4-18.6 °C, whereas *C. rutilans cyanescens* occurs in
31 regions under 1 000 m and adults are active at 15.3-24.0 °C (Hensen et al., 2018). These two
32 subspecies are rollers (telecoprids), i.e., they make dung balls that are buried for feeding or
33 nesting. When nesting, females transform the ball to a 'water drop' shape and put one egg in it

1 (Halffter & Edmonds, 1982; Hernández et al., 2020). Males and females cooperate in parental
2 care of brood balls (Halffter & Edmonds, 1982).

3 After collecting in a field station, specimens of both subspecies were bred in the laboratory
4 at different temperatures. As is often the case, different populations of both subspecies may
5 have a specific but plastic thermal response (Chown & Terblanche, 2006). In this study, we
6 assume that the specific thermal responses of the selected individuals will become apparent
7 under the conditions used along an experimental gradient. The main objective of this study
8 was to compare the influence of temperature on the behaviour, dung ball production, larval
9 development, and adult longevity of *C. rutilans rutilans* and *C. rutilans cyanescens*. We
10 hypothesize that the adult behaviour and development of the specimens differ between the
11 subspecies in agreement with their thermal preferences derived from field data.

12

13 **Materials and methods**

14 **Dung beetle capture for experiments**

15 Dung beetles were captured in two areas of the Santa Catarina State, southern Brazil, as these
16 subspecies do not seem to co-occur (Hensen et al., 2018). The specimens of *C. rutilans*
17 *rutilans* were collected during the summer (end of December 2016) at 1 360 m above sea
18 level in São Joaquim National Park (28°8'28"S, 49°38'13"W), in the municipality of Urubici.
19 The individuals of *C. rutilans cyanescens* were also collected during the summer (December
20 2017 and January 2018) at 250 m a.s.l. in Serra do Tabuleiro State Park (27°44'01"S,
21 48°48'33"W), in the municipality Santo Amaro da Imperatriz. Although the specimens of the
22 two subspecies were collected in different years for logistical reasons, we assume that the
23 effect of this difference is negligible compared with effects of factors studied in the
24 experiments. Dung beetles were sampled several times until we collected enough individuals
25 to conduct laboratory experiments. All unnecessary specimens (from other species) were
26 released in the same location where they had been collected. We used pitfall traps baited with
27 dog faeces to collect twice a day (morning and afternoon) the studied individuals because this
28 bait has proven to be effective in collecting dung beetles (Batilani-Filho & Hernández, 2017).
29 These traps were plastic containers (15 cm diameter, 20 cm deep) buried in the ground up to
30 the rim and closed with a plastic lid containing a hole allowing the entry but preventing the
31 escape of individuals. The traps were protected from rain with a plastic cover approximately
32 10 cm above the container. The traps contained local soil and a piece of dog faeces (ca. 30 g)
33 in the centre. The faeces were previously obtained from dogs not submitted to any medical

1 treatment and were kept under refrigeration at the Biotério Central (Bioterium) of the
2 Universidade Federal de Santa Catarina.

3

4 **Rearing and maintenance under different temperatures**

5 The beetles were transported to the Laboratory of Terrestrial Animal Ecology
6 (LECOTA/UFSC) and maintained at 26 ± 1 °C and L12:D12 photoperiod. They were stored
7 in 5-l plastic pots with soil from the location and were weekly fed with dog faeces until the
8 beginning of the experiments. The individual of *C. rutilans rutilans* (Figure 1A) and *C.*
9 *rutilans cyanescens* (Figure 1B) were individually identified, weighed, sexed, and classified
10 into five age categories (very young, young, mature, old, and very old) according to the tibia
11 degree and clypeus erosion (Tyndale-Biscoe, 1984). Only those specimens classified as young
12 or mature (some tibia spines missing and some erosion in the tarsal spur and clypeus) were
13 used in the experiments to guarantee the sexual maturity of the individuals.

14 Six pairs from each subspecies were allocated per treatment. Each pair is composed of
15 specimens with similar body size (heavy, medium, or light) to minimize the possible effect of
16 body size differences on the measured behavioural and developmental variables. Thus, we
17 organized the 30 pairs by weight, with male and female from each pair with similar weight,
18 and then they were distributed randomly among the treatments. Each pair was kept in a clear
19 plastic container (13 cm diameter, 11 cm deep) with 500 g of soil from the location where
20 each subspecies was collected, as suggested by Villalobos et al. (1998). The soil was
21 previously sifted and heated for 90 s in a microwave at maximum power in order to remove
22 possible pathogenic bacteria and fungi. The containers had a plastic lid with a hole in the
23 middle covered by a tissue that allowed air entry. During the experiment, the soil in the
24 containers was constantly kept moist by periodically spraying it with water. All pairs were fed
25 weekly with 10 g of dog faeces due to their easy collection in the Bioterium, where the dogs
26 are being fed in a standardized and safe way. Further, this type of resource has proven to be a
27 suitable trophic resource for laboratory rearing of dung beetles when compared with the dung
28 of several native mammal species (Hernández et al., 2020).

29 Pairs included in the containers were maintained at five thermal conditions or treatments
30 (15, 20, 25, 30, and 35 °C), and each treatment was repeated 6×; thus, we used a total of 120
31 individuals (2 subspecies × 2 individuals × 6 repetitions × 5 thermal treatments) and 30
32 experimental units per subspecies (6 repetitions × 5 thermal treatments). We selected the
33 minimum temperature (15 °C) considering the lowest environmental mean temperature
34 experienced by the active adults of these two subspecies (Hensen et al., 2018). Beginning with

1 15 °C, treatments increased by 5 °C until a maximum of 35 °C, a value close to the
2 temperature in which one can find active adults of *C. rutilans rutilans* (33.5 °C) and *C.*
3 *rutilans cyanescens* (32.3 °C). 15 °C was maintained in the laboratory with air condition,
4 whereas the four higher temperatures were maintained by placing the containers in four
5 independent electric Titã ACL-5810D showcases (77 × 36.5 × 44.5 cm). Although the
6 experiments for each subspecies were carried out in different years, they started in the same
7 period during the summer: for *C. rutilans rutilans* the experiment started in January 2017 and
8 for *C. rutilans cyanescens* in February 2018. After the experiment (7 weeks of data), we
9 maintained the pairs under the experimental conditions until 90% of the individuals of each
10 subspecies died (around 30 weeks or approximately 4× the experimental period). Thus, the
11 experiment of *C. rutilans rutilans* ended in November 2017 and for *C. rutilans cyanescens* in
12 June 2018.

13

14 **Behavioural activity**

15 Photographs of each pair/container were taken the day the pair was fed. The clear plastic
16 container lids were removed and replaced with glass lids. A Canon T3 camera connected to a
17 laptop was attached to a tripod to take pictures from above. Both subspecies have a diurnal
18 behaviour and were maintained in the laboratory in a L12:D12 day/night cycle (day from
19 07:00 to 19:00 hours). The camera took pictures every 5 min for 3 h (time lapse), beginning at
20 15:00 and finishing at 18:00 hours. Each pair was photographed only 1 day in the experiment,
21 with 40 photos per pair totalizing 1 200 photos (40 photos/pair × 6 pairs/temperature × 5
22 temperature treatments). Because five pairs of *C. rutilans cyanescens* died before they could
23 be photographed, we only analysed 1 000 photographs for this subspecies. Thus, the data
24 finally analysed came from 30 pairs of *C. rutilans rutilans* and 25 pairs of *C. rutilans*
25 *cyanescens*.

26 Sequential images allow us to detect each one of the movements and behavioural patterns
27 of all the individuals. In total 80 responses per pair were analysed. As there were two
28 individuals per container, we registered in each photo what each individual was doing. Thus,
29 we have two behavioural activities per photo except in the case of sexual behaviours. Based
30 on these observations we described the behaviour and classified the behavioural patterns into
31 four behavioural categories: sexual (copulation and parental care), locomotion (move on the
32 surface), alimentation (feed and stay on the food ball), and others (buried) (Table 1). The data
33 of each beetle were divided into six behavioural patterns: copulation (COP), parental care
34 (PCARE), movement on the surface (MOVE), feeding (FEED), staying on the food ball

1 (STAY), and burying (BURY).

2

3 **Dung ball production**

4 The 30 experimental units per subspecies were followed for 8 weeks (7 weeks of data) by
5 counting weekly the food and brood dung balls made by each beetle pair. Additionally, we
6 weighed the balls with a precision balance and measure their size (diameter) with a digital
7 caliper. Food and brood dung balls can be clearly distinguished due to the ‘water drop’ shape
8 of brood balls. The mean weight and diameter of the balls made by each pair were used in the
9 statistical analyses as individual identification of each ball was not possible (balls can be
10 destroyed by a pair and new balls can be built between observation periods). We consider that
11 the data obtained from each pair are statistically independent because their own behaviour
12 does not influence the behaviour of the other pairs (Schank & Koehnle, 2009).

13

14 **Emergence and longevity**

15 The newly emerged specimens in each container were counted and individuals were weighed
16 in order to assess possible differences due to temperature treatments and subspecies identity.
17 Finally, we calculated the number of weeks elapsed until the death of each individual of each
18 subspecies (longevity) to examine whether the average lifetime of the two subspecies differed
19 due to temperature treatments. Although longevity will depend on the age of the individuals at
20 the moment of their capture, we assumed that the random distribution of individuals along the
21 temperature experiments minimized the role of this possible bias.

22

23 **Statistical analysis**

24 A full factorial ANOVA design in General Linear Models was used to estimate the statistical
25 significance of the dependent variables, including temperature treatments (five levels) and
26 subspecies identity (two levels) as explanatory factors. The two main effects (temperature and
27 subspecies) and their interaction were tested ($\alpha = 0.05$), but if the interaction was statistically
28 significant, the main effects were ignored in the interpretation of the results. We used Tukey
29 honestly significant differences (HSD) post-hoc tests after Bonferroni corrections to test for
30 differences in mean values among factor levels. These calculations were done with the
31 software R v.3.2.3 and histogram tools ggplot2 (Wickham & Chang, 2016).

32

33 **Results**

1 **Body weight**

2 The subspecies identity factor was significant for body weight differences ($F_{1,116} = 6.98$, $P =$
3 0.009): where the individuals of *C. rutilans rutilans* are heavier than those of *C. rutilans*
4 *cyanescens* (mean \pm SE = 0.171 ± 0.039 vs. 0.153 ± 0.035 g, both $n = 60$). The sex factor does
5 not significantly explain body weight differences ($F_{1,116} = 0.61$, $P = 0.44$).

7 **Ethogram**

8 The predominant behaviour observed at all temperature treatments in both subspecies was
9 staying buried under the ground (BURY), which was observed in more than half of all
10 occasions, and in more than 80% of occasions at 20 °C, independent of subspecies identity
11 (Table 2). Individuals moving on the ground (MOVE) or feeding in the dung (FEED) were
12 observed in ca. 21 and 8% of the occasions, respectively. The other behavioural patterns
13 occurred much less frequently (Table 2). The few statistically significant relationships suggest
14 that *C. rutilans cyanescens* would move more on the soil surface, regardless of temperature,
15 and that all the specimens feed more at higher temperatures (Table 2).

17 **Food and brood ball production**

18 The number of food balls per pair oscillates from 0 to 37 (mean \pm SE = 12.5 ± 2.0), whereas
19 the number of brood balls is lower (6.2 ± 4.4 ; range: 0-24). The number of food balls did not
20 differ statistically between the two subspecies or between temperatures (Table S1). However,
21 temperature was found to have a significant effect on the number of brood balls (Table S1).
22 Tukey's HSD tests indicated that the number of brood balls at 15 °C is lower ($P = 0.02$) than
23 the numbers at higher temperatures (30°C) independently of the subspecies (Figure 2)..

25 **Weight and size of food and brood balls**

26 The correlation between the weight and diameter of food and brood balls is very high (both r
27 = 0.95 , $P < 0.001$). Weight and diameter of food balls differ between temperatures and
28 subspecies (Table S2). Food balls are smaller and lighter for *C. rutilans cyanescens*, as well as
29 in extreme temperatures (15 and 35°C; Tukey's HSD tests: $0.02 > P > 0.002$; Figure 3A,B). For
30 brood balls the temperature*subspecies interaction is always statistically significant (Table
31 S2), indicating that the responses of weight and diameter of the dung balls to temperature
32 treatments depends on the subspecies (Figure 3C,D). Interestingly, *C. rutilans rutilans* brood
33 balls seem to be bigger and heavier at lower temperatures, whereas those of *C. rutilans*
34 *cyanescens* remain practically unchanged along the temperature treatments.

1

2 **Emerged adults and longevity**

3 A total of 28 new adults emerged from the brood balls of *C. rutilans rutilans* and 15 from *C.*
4 *rutilans cyanescens*. The first *C. rutilans rutilans* emerged during the 7th week at 30 °C
5 whereas the last one emerged in the 16th week at 15 °C. The first *C. rutilans cyanescens*
6 emerged during the 5th week at 30 °C, the last during the 12th week at 25 °C. The number of
7 newly emerged individuals did not differ between subspecies, but was highly dependent on
8 temperature (Table S3). In general, the emergence of new individuals increased up to 30 °C,
9 and decreased drastically at 35 °C, regardless of the subspecies (Figure 4A).

10 The weight of the newly emerged individuals differed among temperatures and was highly
11 dependent on subspecies (Table S3); new adults of *C. rutilans rutilans* were heavier than
12 those of *C. rutilans cyanescens*. Tukey's HSD tests indicated that the emerged individuals
13 were significantly heavier at 20 °C than at higher temperatures (Figure 4B).

14 The time required for the emergence of new adults differed between temperature
15 treatments (Table S3) but not between subspecies. Thus, new adults need more time to
16 emerge under low temperatures (15 and 20 °C; Tukey's HSD tests: $P < 0.001$; Figure 4C).
17 Finally, the mean (\pm SE) longevity of both subspecies is 11.0 ± 1.3 weeks, oscillating from 1
18 to 30 weeks. Interestingly, the temperature*subspecies interaction is highly significant in
19 explaining the longevity of wild-caught adults (Table S3); *C. rutilans rutilans* live longer than
20 *C. rutilans cyanescens* at all the temperatures, except 35 °C (Figure 4D). Thus, the longevity
21 of *C. rutilans rutilans* decreases with increasing temperature, whereas longevity of *C. rutilans*
22 *cyanescens* seems to be temperature independent (Figure 4D).

23

24 **Discussion**

25 Our results indicated that the two subspecies differ in their reproductive strategy depending on
26 the environmental temperature, as well as in their larval development and longevity, matching
27 their regional occurrences. We found no significant behavioural differences between the
28 subspecies, perhaps due to the low sample sizes. Below 25 °C, the number of dung balls for
29 breeding in *C. rutilans cyanescens* was low. This is in agreement with this subspecies'
30 distribution – it occurs under warmer conditions (Hensen et al., 2018). Furthermore, at low
31 temperatures the weight and diameter of the brood balls were significantly higher in *C.*
32 *rutilans rutilans* than in *C. rutilans cyanescens*, as were the weight (but not the number) of
33 newly emerged adults and the longevity of parental specimens at the lowest temperature

1 considered. All these results support thermal segregation between the two subspecies,
2 reinforcing the likelihood that they could be considered as different species (Hensen et al.
3 2018). Considering that genetic isolation is the main factor generating the differences of any
4 kind able to classify them as different species (De Queiroz, 2007), further genetic analyses
5 may confirm their separation as different species.

6 Our results also corroborate the role of temperature in determining body size,
7 developmental time, or brood ball weight. In ectotherms, temperature affects the rate of
8 development and the age at which an individual reaches its body size (Atkinson & Sibly,
9 1997; Angilletta & Dunham, 2003). Our results show that *C. rutilans rutilans* adults are larger
10 than those of *C. rutilans cyanescens*. In a revision, Atkinson (1994) showed that in many
11 cases the body size of insects is reduced with an increase in temperature (examples in Diptera,
12 Hemiptera, Hymenoptera, and Lepidoptera), but this pattern is variable in Coleoptera. Simple
13 explanations trying to establish a general temperature-size rule in ectotherms are insufficient,
14 as in many special cases the mechanisms involved may be complex (Atkinson & Sibly, 1997;
15 Angilletta & Dunham, 2003).

16 In addition, the behaviour of the two subspecies may be unequally influenced by
17 temperature. *Canthon rutilans rutilans* adults remained buried in the ground longer than
18 adults of *C. rutilans cyanescens*, which moved on the ground more. This difference may be
19 related to environmental temperature, as the soil has a temperature-buffering capacity and *C.*
20 *rutilans rutilans*, the characteristically ‘cold’ subspecies, may avoid low temperatures by
21 staying underground (Lobo et al., 2019). Temperature variation did not influence the feeding
22 patterns in the two subspecies in contrast to the influence of temperature on reproductive
23 behaviour. The production of brood balls depends on temperature in both subspecies. *Canthon*
24 *rutilans rutilans* produce larger and bigger brood balls (more faeces inside) at lower
25 temperatures. This may be another adaptation to living at lower temperatures as larvae may
26 need more time to develop under these conditions. Dung beetles can adjust the brood ball size
27 according to dung quality (Kishi, 2014) and adult body size (Hunt & Simmons, 2000, 2002).
28 Females of *C. rutilans rutilans* produced fewer brood balls at lower temperatures than at
29 higher temperatures, but they were bigger. The new adults were heavier at lower
30 temperatures. Temperature is also known to influence larval development time: at lower
31 temperatures the larvae develop slower.

32 The first individual of both subspecies emerged at the same temperature. However, new
33 adults of *C. rutilans rutilans* needed 7 weeks to complete their life cycle and new adults of *C.*
34 *rutilans cyanescens* only 5 weeks. The last individual to emerge was after 4 months at the

1 lowest temperature for *C. rutilans rutilans*, and after 3 months at 25 °C for *C. rutilans*
2 *cyanescens*, reinforcing the adaptation of *C. rutilans rutilans* towards cold conditions. Both
3 subspecies produced brood balls at the lowest temperature, although fewer, but only the larvae
4 of *C. rutilans rutilans* were able to complete their development..

5 Some studies with *C. rutilans cyanescens* showed that adults, feeding on dog faeces at 25
6 °C, had a F1 with a larval development cycle that lasts 33 ± 4 days on average (Hernández et
7 al., 2020). The most studied species of this genus is *Canthon cyanellus cyanellus* LeConte,
8 whose ideal breeding temperature under laboratory conditions has been found to be 27 ± 1 °C
9 in Mexico (Favila et al., 2005). The development time increases considerably at low
10 temperatures in most insects (Ratte, 1984), and there are no differences between the number
11 of larval stages at different temperatures, only the time that they need to develop and to reach
12 the same size changes (Sehnal, 1991). The survival of males from *C. rutilans cyanescens*
13 reaches more than three months (Wuerges & Hernández, 2020), but we observed that
14 temperature did not affect its survival. Nevertheless, the survival of *C. rutilans rutilans* was
15 almost three times less at high temperatures. This result demonstrates the relationship between
16 temperature and mortality due to aging; with an increase in temperature there is greater
17 molecular damage, and therefore mortality will increase (Callow, 1978).

18 Differential adaptations to the climatic conditions along elevational gradients can lead to
19 allopatric speciation (Janzen, 1967; Ghalambor et al., 2006; Mittelbach et al., 2007).
20 Currently, *C. rutilans rutilans* and *C. rutilans cyanescens* are considered two subspecies due
21 to their adult morphological similarity. A recent study (Hensen et al., 2018) showed that the
22 two subspecies are spatially segregated, and also that they inhabit under conditions that differ
23 in environmental temperature. In the current study, we showed that environmental
24 temperature influenced both subspecies differently: *C. rutilans rutilans*, occurring at high
25 elevations, has a more variable response to environmental temperature than *C. rutilans*
26 *cyanescens*, which occur under warmer conditions. Thus, our results support that these two
27 taxa may be considered different species. Further genetic analyses would help to confirm this
28 hypothesis.

29

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7

8 **Figure captions**

9 **Figure 1** Adults of *Canthon rutilans* subspecies: (A) characteristically red *C. rutilans rutilans*
10 and (B) blue *C. rutilans cyanescens*.

11

12 **Figure 2** Number of (A) food balls and (B) brood balls made by pairs of *Canthon rutilans*
13 *rutilans* (white) and *C. rutilans cyanescens* (grey) during 7 weeks of laboratory experiments
14 at 15-35 °C. The star and the central line in each box represent the mean and median,
15 respectively. The boxes represent the first and third quartile values, the vertical lines represent
16 the 5th and 95th percentiles, and the dots are outliers.

17

18 **Figure 3** (A, B) Weight and (C, D) diameter of (A, C) food balls and (B, D) brood balls made
19 by pairs of *Canthon rutilans rutilans* (white) and *C. rutilans cyanescens* (grey), during 7
20 weeks of laboratory experiments at 15-35 °C. The star and the central line in each box
21 represent the mean and median, respectively. The boxes represent the first and third quartile
22 values, the vertical lines represent the 5th and 95th percentiles, and the dots are outliers..

23

24 **Figure 4** (A) Number, (B) weight (g), and (C) time of emergence (weeks) of F1 individuals,
25 and (D) adult longevity (weeks) of wild-caught individuals of *Canthon rutilans rutilans*
26 (white) and *C. rutilans cyanescens* (grey) at 15-35 °C. The star and the central line in each
27 box represent the mean and median, respectively. The boxes represent the first and third
28 quartile values, the vertical lines represent the 5th and 95th percentiles, and the dots are
29 outliers In panels B and C, the boxplot at 15 °C belongs to *C. rutilans rutilans*.

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1 **Supporting Information**

2 Additional Supporting Information may be found in the online version of this article.

3

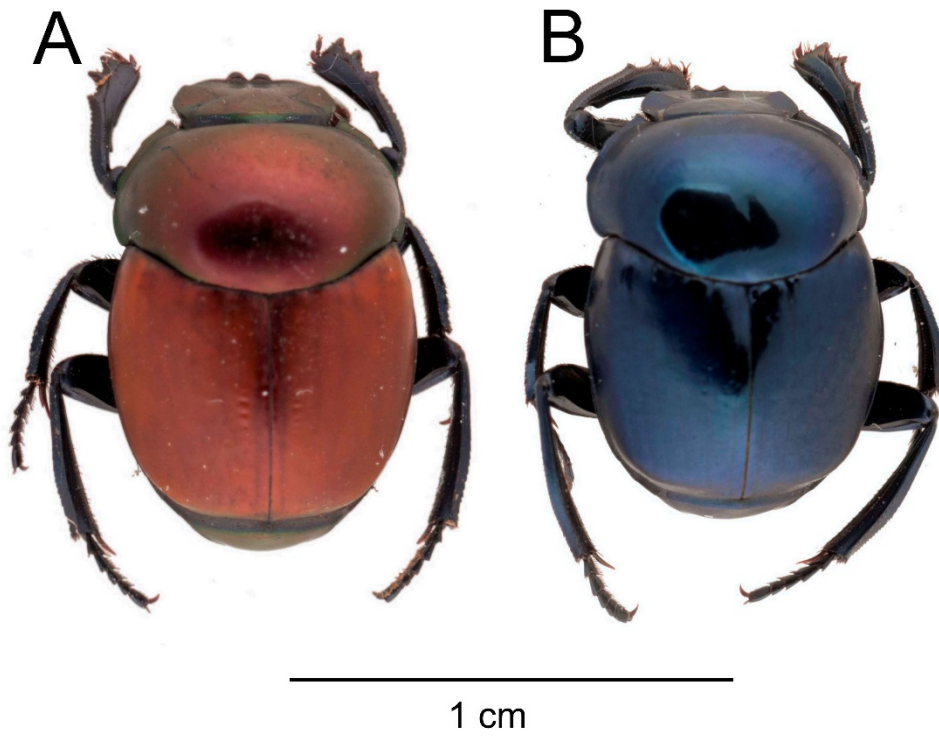
4 **Table S1** Results from a GLM full factorial design accounting for the number of food and
5 brood balls, including temperature treatments (five levels) and subspecies identity (two levels)
6 as explanatory factors. $R^2 \times 100\%$ is the percentage of total variability explained by the
7 complete model

8

9 **Table S2** Results from a GLM full factorial design for the weight and diameter of food and
10 brood balls, including the treatments of temperature (five levels) and subspecies identity (two
11 levels) as explanatory factors. $R^2 \times 100\%$ is the percentage of total variability explained by
12 the complete model

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14 **Table S3** Results from a GLM full factorial design for the number, weight, and emergence
15 time of F1 and for adult longevity, including the temperature treatments (five levels) and
16 subspecies identity (two levels) as explanatory factors. $R^2 \times 100\%$ is the percentage of total
17 variability explained by the complete model

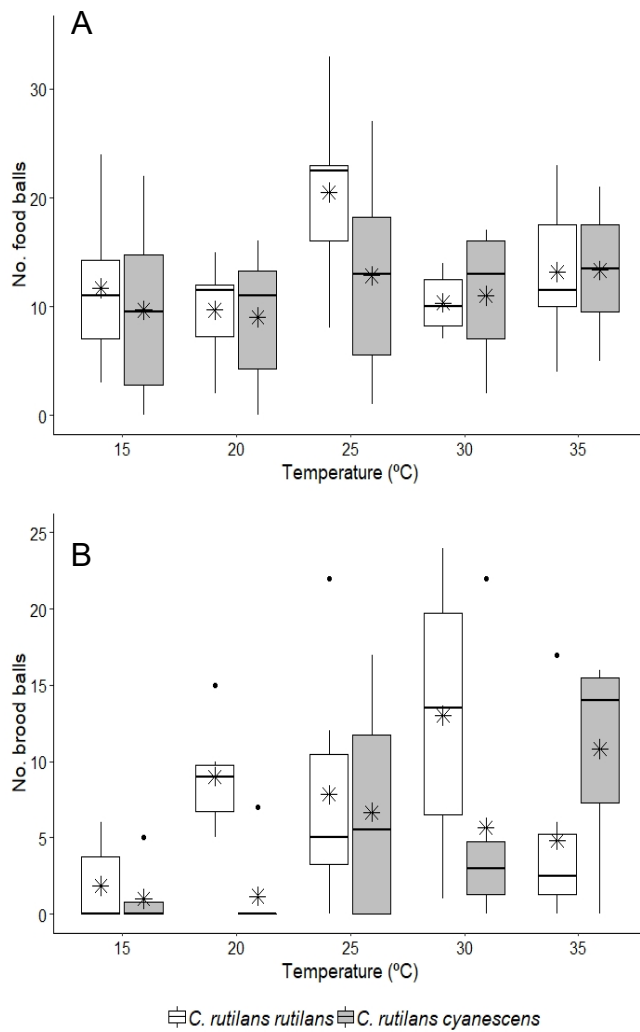


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Figure 1

1 **Figure 2**

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3

C. rutilans rutilans *C. rutilans cyanescens*

4 -all lettering in figures should be ARIAL

5 -move the text labels along the vertical axes a bit away from the axes please

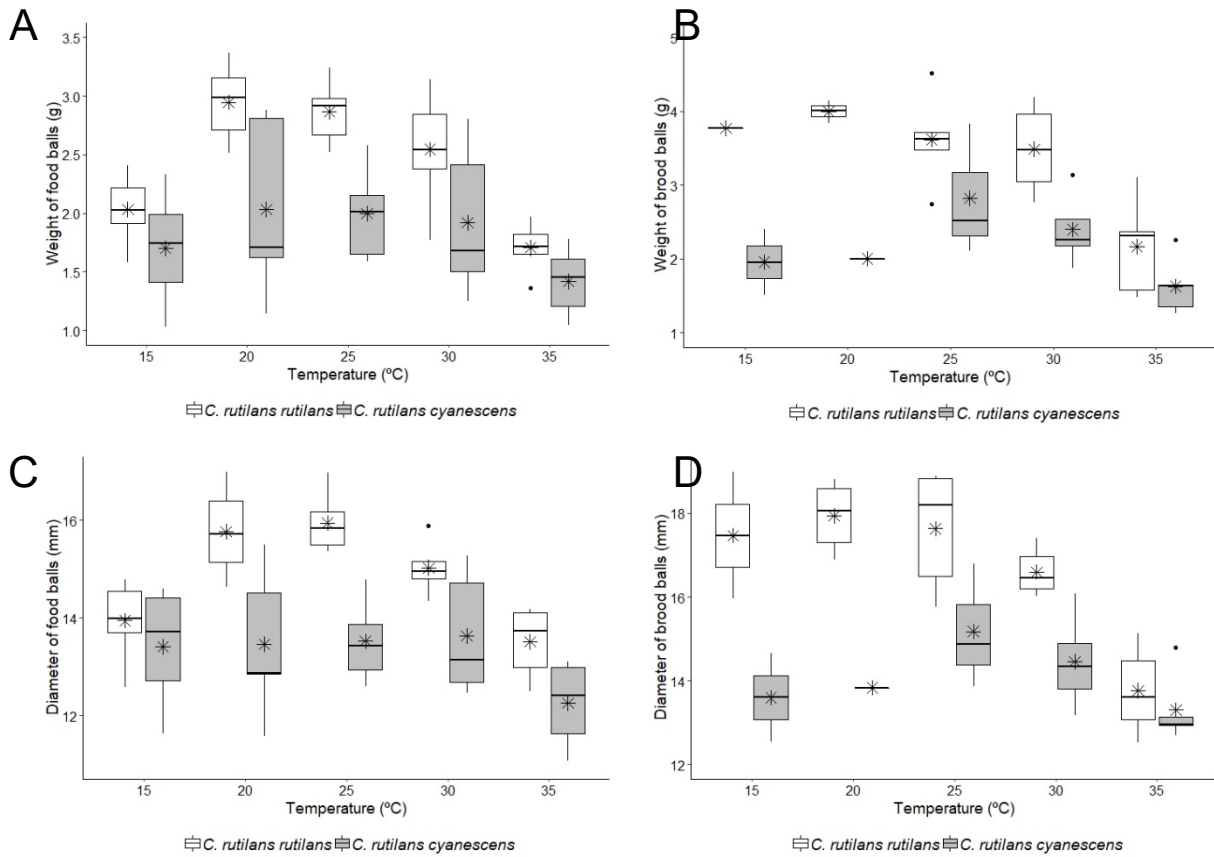
6 -remove 'Temperature (°C)' from the top panel

7 -remove the legend <info is already in the caption>

8 Put caps on the whiskers, about half the width of the box

1 **Figure 3**

2



3 -all lettering in figures should be ARIAL

4 -move the text labels along the vertical axes a bit away from the axes please

5 -remove 'Temperature (°C)' from the top two panels; move the lower two panels a bit up

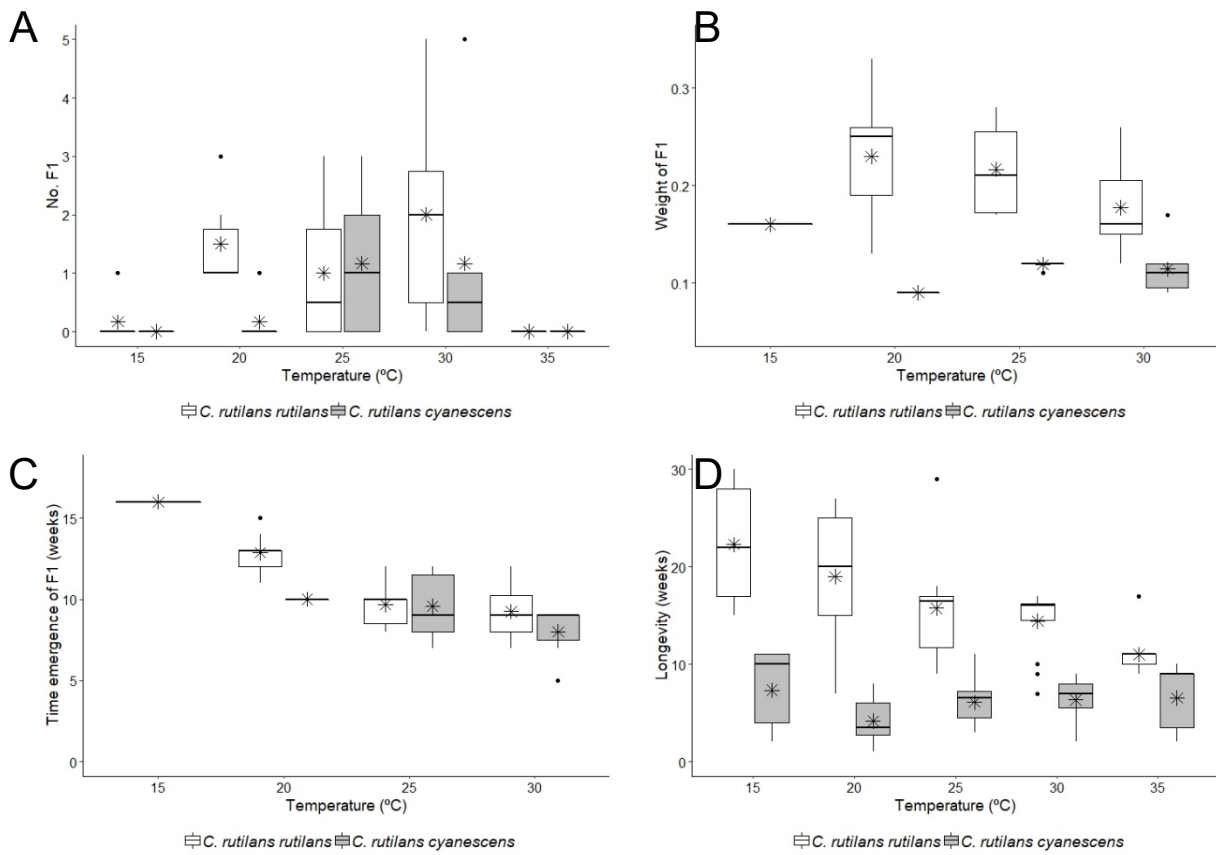
6 -remove the four legends <info is already in the caption>

7 Put caps on the whiskers, about half the width of the box

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1 **Figure 4**

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3 -all lettering in figures should be ARIAL

4 -move the text labels along the vertical axes a bit away from the axes please

5 -remove 'Temperature (°C)' from the top two panels; move the lower two panels a bit up

6 -remove the four legends <info is already in the caption>

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8 Put caps on the whiskers, about half the width of the box

1 **Table 1** Ethogram of behavioural categories observed in *Canthon rutilans* under laboratory
 2 conditions

Behavioural categories	Behavioural patterns	Description of behaviour
Sexual	Copulation (COP)	Male adheres to the female in the dorsal part
	Parental care (PCARE)	Individual is above the brood ball, dislocates/rolls the brood ball, buries the brood ball
Locomotion	Move on the surface (MOVE)	Individual walks along the side of the case, tries to climb on the case wall, is on the soil surface
Alimentation	Feed (FEED)	Individual feeds on faeces or food balls
	Stay on the food ball (STAY)	Individual is on the food ball
Others	Buried (BURY)	Individual is hidden in the soil

3

1 **Table 2** Behavioural patterns (% of time spent) in pairs of the two subspecies of *Canthon*
 2 *rutilans* in the laboratory at 15-35 °C and results of two-way-ANOVA

Subspecies	T (°C)	COP	PCARE	MOVE	FEED	STAY	BURY
<i>C. rutilans rutilans</i>	35	6.67	3.33	19.17	19.58	4.37	46.88
	30	0	4.37	10.00	12.92	14.38	58.33
	25	0	12.92	8.54	3.54	6.25	68.75
	20	0	0	11.67	5.21	0	83.12
	15	0.83	0.21	21.88	2.92	10.83	63.33
<i>C. rutilans cyanescens</i>	35	0	0.25	21.50	15.00	8.25	55.00
	30	3.50	15.00	31.50	5.25	18.00	26.75
	25	0	14.25	32.25	5.75	0	47.75
	20	0	0	13.12	0.63	0.31	85.94
	15	0.83	0	42.71	5.83	0	50.63
Temperature (F _{4,45})		1.27 ns	2.29 ns	1.94 ns	3.40*	2.11 ns	4.11**
Subspecies (F _{1,45})		0.33 ns	0.22 ns	9.17**	0.68 ns	0.25 ns	2.65 ns
Temperature*subspecies (F _{4,45})		2.25 ns	0.40 ns	1.13 ns	0.56 ns	0.65 ns	1.20 ns

3 COP, copulation; PCARE, parental care; MOVE, movement on the surface; FEED, feeding;

4 STAY, staying on the food ball; BURY, burying in the soil.

5 *0.01<P≤0.05; **P≤0.01.

6

1 **Supporting Information**

2 **Table S1** Results from a GLM full factorial design accounting for the number of food and
3 brood balls, including temperature treatments (five levels) and subspecies identity (two levels)
4 as explanatory factors. $R^2 \times 100\%$ is the percentage of total variability explained by the
5 complete model

	Temperature	Subspecies	Temperature*subspecies	$R^2 \times 100\%$
No. food balls	$F_{4,50} = 1.58$ P = 0.19	$F_{1,50} = 0.27$ P = 0.61	$F_{4,50} = 1.04$ P = 0.40	17.7
No. brood balls	$F_{4,50} = 2.92$ P = 0.03	$F_{1,50} = 1.93$ P = 0.17	$F_{4,50} = 2.48$ P = 0.06	32.0

6

1 **Table S2** Results from a GLM full factorial design for the weight and diameter of food and
 2 brood balls, including the treatments of temperature (five levels) and subspecies identity (two
 3 levels) as explanatory factors. $R^2 \times 100\%$ is the percentage of total variability explained by
 4 the complete model

	Temperature	Subspecies	Temperature*subspecies	$R^2 \times 100\%$
Weight food balls	$F_{4,48} = 7.90$ P<0.001	$F_{1,48} = 27.73$ P<0.001	$F_{4,48} = 1.59$ P = 0.19	57.8
Weight brood balls	$F_{4,30} = 15.14$ P<0.001	$F_{1,30} = 30.78$ P<0.001	$F_{4,30} = 3.01$ P = 0.03	77.5
Diameter food balls	$F_{4,48} = 5.95$ P<0.001	$F_{1,48} = 38.50$ P<0.001	$F_{4,48} = 2.36$ P = 0.07	59.9
Diameter brood balls	$F_{4,30} = 15.35$ P<0.001	$F_{1,30} = 33.33$ P<0.001	$F_{4,30} = 2.80$ P = 0.04	77.9

5

1 **Table S3** Results from a GLM full factorial design for the number, weight, and emergence
 2 time of F1 and for adult longevity, including the temperature treatments (five levels) and
 3 subspecies identity (two levels) as explanatory factors. $R^2 \times 100\%$ is the percentage of total
 4 variability explained by the complete model

Parameters	Temperature	Subspecies	Temperature*subspecies	$R^2 \times 100\%$
No. F1	$F_{4,50} = 4.62$	$F_{1,50} = 2.39$	$F_{4,50} = 1.01$	33.3
	$P = 0.003$	$P = 0.13$	$P = 0.41$	
Weight of F1	$F_{3,35} = 4.49$	$F_{1,35} = 30.09$	$F_{2,35} = 1.33$	56.9
	$P = 0.009$	$P < 0.001$	$P = 0.28$	
Time emergence of F1	$F_{3,36} = 17.70$	$F_{1,36} = 3.32$	$F_{2,36} = 1.22$	62.0
	$P < 0.001$	$P = 0.08$	$P = 0.31$	
Longevity	$F_{4,101} = 7.33$	$F_{1,101} = 181.72$	$F_{4,101} = 7.01$	70.3
	$P < 0.001$	$P < 0.001$	$P < 0.001$	

5