

Conservation of *Lygodactylus williamsi* (Reptilia: Squamata) in the Kimboza Reserve, Tanzania, and its breeding in Liberec Zoo, Czech Republic

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Abstract. One of the main roles of modern zoos is the conservation of endangered animal species, primarily by keeping populations of them in captivity (*ex situ* conservation) and also by protecting these animals in their natural environment (*in situ* conservation). Liberec Zoo meets both of the aforementioned roles for several of the species kept, especially birds of prey. However, the zoo has never focused on the *in situ* and *ex situ* conservation of an endangered reptile species. The limiting factor in the past has often been the size of the exhibits and a lack of new spaces. The turquoise dwarf gecko (*Lygodactylus williamsi* Loveridge, 1952) was an ideal candidate for this due to its small size. The aim is to become part of a coordinated captive breeding programme, while also getting involved in turquoise dwarf gecko conservation in the wild. This Tanzanian *in situ* project is underway in the tropical lowland forest of the Kimboza Reserve. Here, the turquoise dwarf gecko is intricately tied to the screw pine (*Pandanus rabaiensis*), which is being displaced by the non-native and fast-growing Spanish cedar (*Cedrela odorata*). Other no less significant threats to the local gecko population include fires caused by slash-and-burn farming, illegal logging and mineral extraction, and the illicit capture of specimens for the pet trade. The project is focused on protecting the turquoise dwarf gecko's habitat with the support of local communities. By means of various educational and fundraising activities, project members are trying to secure the financial means necessary to implement sub-activities such as creating firebreaks to prevent the spread of fires in the forest, funding forest rangers and their equipment, as well as clearing the forest of invasive trees. There is also an emphasis on environmental education, training and awareness raising, not only for students in schools in Tanzania but also in Europe by means of the zoological institutions involved.

Key words. *Ex situ* conservation, *in situ* breeding, reserve, *Pandanus*, gecko.

INTRODUCTION

The turquoise dwarf gecko (*Lygodactylus williamsi* Loveridge, 1952) is a small lizard species that currently occurs in just two locations, both fragments of the tropical forest in eastern Tanzania. These are the Ruvu and Kimboza Forest Reserves, with a total area of approximately 20 km². The Kimboza Reserve lies at the foothills of the Uluguru Mountains (eastern part) and, due to deforestation, a fragment of just 4.05 km² forest now remains (Kilawe et al. 2022). The Ruvu River flows through Kimboza and is an important source of water not only for the local inhabitants but also for the flora and fauna. Despite its small size, it has a high biodiversity of animal and, in particular, plant species (Rodgers et al. 1983, Kacholi 2013). Kimboza is a lowland forest with the highest number of recorded bird species in East Africa, a total of 82 species have been described (Doggart et al. 2001). Scientists have classified 22 amphibian species and 34 reptile species in both reserves, some of which have been discovered for the first time (Flecks 2022).

The turquoise dwarf gecko is an endemic reptile species that inhabits a relatively small, fragmented area where it lives exclusively on the screw pine (*Pandanus rabaiensis*) which must have

sufficiently long leaves (Flecks et al. 2012). It prefers large trees and lives at a height of 2–5 m (Figs. 1–2). This tree species occupies 17.6% of the area in the reserve and only grows on limestone bedrock (Weinsheimer & Flecks 2010). The characteristics described above mean the turquoise dwarf gecko is highly vulnerable to extinction. Due to long-term, large-scale disturbances to its natural habitat, the turquoise dwarf gecko has been on the International Union for Conservation of Nature’s (IUCN) Red List of Threatened Species in the category “Critically Endangered” since 2012. In the Convention on International Trade in Endangered Species (CITES), it falls under Appendix I, which includes animal species threatened with extinction. International trade in wild-caught specimens is prohibited. A project to protect the turquoise dwarf gecko from the negative impacts that are primarily caused by human activities has been underway in the Kimboza Reserve since 2020. The main threats include deforestation caused by fires from slash-and-burn farming and illegal logging for timber, gold and gems. A similarly serious problem is the spread of the non-native Spanish cedar (*Cedrela odorata*) in the forest, this being at the expense of the native screw pine (*Pandanus rabaiensis*). The geckos are also illegally caught for the pet trade, mainly due to demand from private breeders in Europe and the USA. Conservation activities in the Kimboza Reserve involve local communities under the leadership of Dr. C. J. Kilawe of the Sokoine University of Agriculture. Dr. Heike Maisch from Erfurt Zoopark in Germany coordinates the project in Europe. Apart from the afore-mentioned zoopark, the project is financially supported by the Zoological Society for the Conservation of Species and Populations (Zoologische



Fig. 1. The turquoise dwarf gecko is bound to the pandan tree (*Pandanus rabaiensis*) in the Kimboza reserve. It prefers taller trees with leaves up to 1 m long (photo by C. J. Kilawe).



Fig. 2. The adult male of turquoise dwarf gecko is blue in color with black stripes on the throat. Males are territorial and guard their territory, the pandan tree (photo by C. J. Kilawe).

Gesellschaft für Arten- und Populationsschutz, ZGAP) and ViVe (Vivaristische Vereinigung e.V.) the German exotic animal breeders' association.

The project's main objectives are to preserve the gecko's natural habitat and to prevent it from being illegally caught. The following sub-activities are intended to contribute to the achieving these objectives. The first is to create and regularly maintain firebreaks in the forest. The next is to clear the forest of the invasive Spanish cedar and provide funding for forest rangers to prevent poaching in the reserve (Figs. 3–4). So far, Spanish cedar has been cleared from 50 ha of forest in the reserve. It was also possible to clear 15 km of firebreaks to prevent the spread of fires and another 5 km of firebreaks have been recently created under the leadership of Dr. Kilawe. Eight men chosen from local communities are now being trained under the guidance of rangers from the Tanzania Forest Service Agency (TFS) and together they have conducted 36 patrols which have reduced illegal logging in the reserve. In the future, conservation activities should also focus on planting indigenous trees in the reserve and providing additional income opportunities for local communities from ecotourism and the sale of honey from apiaries.

One very important aspect of the project is likewise educating the public, e.g., students in schools, and not just in the gecko's homeland but also abroad. This is where the participating zoos play an important role by educating their visitors, for example during guided tours, holding public events, information panels or via websites and social networks. Liberec Zoo is the only Czech zoo to be involved in the project so far, having become involved at the end of 2020. Promotional activities for the project involve not only the small exhibition of turquoise dwarf geckos and information boards in the tropics pavilion, but also, for example, handing out worksheets for young visitors to the zoo.



Fig. 3. One of the rangers creating a gap against the spread of a forest fire in the Kimboza Reserve (photo by C. J. Kilawe).



Fig. 4. A very important activity of the conservation project in the Kimboza reserve is the clearing of the forest from the invasive cedrela tree, which is rapidly spreading through the forest and displacing the original and vital pandanus for the geckos. Cedrela is a popular fuel and construction wood. Students from the local Sokoine University are also involved in clearing the forest of non-native cedrela (photo by C. J. Kilawe).

Part of the effort to save the turquoise dwarf gecko on our planet is to breed it in captivity in conservation institutions, which serve as a backup if the worst-case scenario, the extinction of the species in the wild, were to occur. The European Association of Zoos and Aquaria (EAZA) has established a European Studbook (ESB) for the species. The aim is to coordinate international cooperation to ensure that a viable and healthy population of turquoise dwarf geckos can be set up in captivity. Erfurt Zoo has been successfully breeding captive turquoise dwarf geckos for a long time. Thanks to the cooperation with Erfurt's curator, Heike Maisch, it was possible to obtain an adult female and male gecko from Leipzig Zoo and accommodate them in the Liberec Zoo (Figs. 5 and 6).



Fig. 5. Exhibition terrarium for the turquoise dwarf geckos in the tropics pavilion of the Liberec Zoo (photo by P. Hnidová).

MATERIALS AND METHODS

A custom-made terrarium was built for the turquoise dwarf geckos. Instead of screw pines it had commonly available *Sansevieria* plants as well as bamboo and a cork branch (Fig. 5). The back wall of this small display consists of a background imitating a rock. A substrate for tropical terrariums with a mixture of sand is used as litter. A drainage layer is placed under the substrate. The lighting, installed above the stainless-steel mesh ceiling, consists of a 35 W halogen bulb (12 h/day) and a 5.0 15 W UVB lamp (4 h/day). The geckos' background area then has a 35 W UVB lamp. The terrarium is usually kept around 24–28 °C during the day, 34–36 °C under the heat lamp. At night, the temperature drops a few degrees lower to 18–20 °C. The humidity in the terrarium is usually between 50–70% during the day and up to 90% at night.

RESULTS

For the first fortnight after arrival, both adults were timid and spent most of the day hidden among the plants. However, they quickly got used to the presence of visitors and keepers. Nevertheless, the keepers' great excitement at the arrival of the new, critically endangered reptile was soon replaced by a modicum of stress. The geckos' acclimatization was also accompanied by a desire to explore the entire pavilion environment, and they tried to escape from their quarters at every opportunity. The fact that the turquoise dwarf geckos are thriving in Liberec is testified to by the fact that less than four months after their arrival at the zoo, the first mating took place and the female laid her first clutch of two eggs.

After 20 days she laid two more. All the eggs were glued to the terrarium wall in a place where the temperature is 24–26 °C during the day and 18–20 °C at night. Given the fact that the



Fig. 6. An adult male of the turquoise dwarf gecko kept in a terrarium in the tropics pavilion of the Liberec Zoo (photo by T. Hnída).

eggshells are very fragile, it was not possible to transfer them to the incubator and there was no interference with the breeding.

In the case of incubation in an incubator, the sex of the turquoise dwarf geckos' young can be influenced by regulating the temperature. According to Heike Maisch, females hatch at 24 °C and males hatch if the temperature is set 2 degrees higher. Thus, the clutches were a surprise, which was only revealed a few months later. The first clutch hatched two young after 55 days, unfortunately the next clutch was not successful.

The offspring were left in the parents' terrarium for the next four months to observe their behaviour within the family. From the very beginning, the parents did not show any predatory or agonistic behaviour towards their offspring. The young often kept close to their mother. At 120 days after hatching, it was decided to separate the juveniles from the adults as they had already started to change into the adult colours. One offspring, the male, acquired the blue colour typical for a male and the other juvenile retained the bronze female coloration.

After a break of almost a year, it is expected that there will be another clutch, as the adult pair was observed mating less than 20 days ago. It is sincerely hoped that the breeding will once again be successful and, as in the case of the first offspring, it will be possible to enrich the population of turquoise dwarf geckos bred in captivity in other zoos involved in the coordinated breeding of this species.

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Intraspecific variation of breathing rate during stress response in *Microtus arvalis* (Mammalia: Rodentia)

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Abstract. Recently, personality traits have been determined in the common vole on the basis of behavioural tests. Both bold as well as shy individuals have been recorded. The aim of the present study was to verify whether variations in the behavioural manifestations in the common vole are also accompanied by variations in the values of usually monitored stress parameters. This study investigated variations in the breathing rate of the common vole, *Microtus arvalis* (Pallas, 1778), and whether these variations are consistent with regard to repetition and stress state. We also investigated possible relationships between breathing rate variation and behavioural personality traits of the tested individuals. Breathing rate values were measured using a device consisting of a respirometric chamber connected to a sensitive pressure sensor and an oscilloscope. Resting breathing rate (RBR) and stress breathing rate (SBR) were measured, and the percentage of stress breathing increase was calculated. The locomotor activity of the observed voles in the Open Field test was used as a behavioural personality trait. It was found that the common vole shows consistent inter-individual differences in breathing rate in all measured parameters. A very close relationship between the individual SBR value and the relative increase of the stress breathing with the personality locomotor trait of the tested animals was demonstrated. Higher values of SBR and stress increase percentage were measured in individuals that appeared to be “shy” in the Open Field test (i.e. anxious animals easily subject to stress). The relationship points to a connection between behavioural and physiological traits, which corresponds with the concept of “coping style” and the distinction between proactive and reactive individuals.

Key words. Common vole, *Microtus arvalis*, breathing rate, stress value, repeatability, coping style.

INTRODUCTION

Behavioural intraspecific differences are not only a manifestation of random variation around the adaptive value of a trait (Carere & Locurto 2011), but a reflection of the close relationship between the behavioural profile of an individual and its life strategy (life-history) (see e.g., Wilson 1994, Réale et al. 2000). Intraspecific behavioural differences that are consistent over time, across situations and contexts are usually referred to as personality. From the point of view of behavioural ecology, two concepts of personality are currently accepted (Réale et al. 2007, 2010). Animal personality in the broad sense is defined as consistent inter-individual differences in a selected type of behaviour e.g., reaction to a predator (Réale 2007). This concept is most often used in studies dealing with the effect of selection on evolutionarily crucial behaviours, e.g., in response to a predator (bold-shy trait/dimension) or in obtaining food (fast-slow trait/dimension).

On the other hand, the narrow sense personality concept, Réale (2007) works with sets of mutually correlated behaviours (personality traits/dimensions), which together create a multidimensional characteristic of the psychological properties of each individual. This concept approaches the concept of personality in human psychology (Pervin & John 1997) and in physiological studies

(referred to here as “coping style”, Koolhaas et al. 1999). It is defined as a set of behavioural and physiological responses to stress that are unique to a given individual and whose nature is consistent over time and across different situations (de Ruiter et al. 1992, Sgoifo et al. 1996, Koolhaas et al. 1999). This latter concept has so far been applied to various animal species, such as the rat, *Rattus norvegicus* (Berkenhout, 1769), the house mouse, *Mus musculus* Linnaeus, 1758, the wild pig, *Sus scrofa* Linnaeus, 1758, and the rhesus macaque, *Macaca mulatta* (Zimmermann, 1780) – see the overview by Koolhaas et al. (1999).

In general, proactive rodent individuals are characterised by lower reactivity of the hypothalamus-pituitary-adrenal axis (hereafter referred to as the HPA axis), manifested by a low level of glucocorticoids in the plasma during stress and higher sympathetic reactivity expressed by a high level of catecholamines during stress. In contrast, reactive individuals show higher HPA axis reactivity and higher parasympathetic reactivity.

In a selection study on domestic mice, *Mus musculus domesticus* Schwarz et Schwarz, 1943, individuals were selected on the basis of attack latency into the lines “short attack latency” (SAL, fast attacking) and “long attack latency” (LAL, slow attacking, Benus et al. 1989). SAL individuals were more aggressive, explored new environments faster, but were also more prone to routine behaviour. LAL individuals, on the other hand, were less aggressive, slower to explore new environments, but more attentive to details and more plastic in behaviour (Benus et al. 1990). SAL and LAL mice have also been found to differ in testosterone levels, with SAL individuals showing higher levels than LAL individuals (de Ruiter et al. 1992). The link between personality and physiological characteristics is also demonstrated by a study conducted on a wild population of alpine marmots, *Marmota marmota* (Linnaeus, 1758), which found a positive relationship between interest in new stimuli and respiratory and heart rates (Ferrari et al. 2013).

During stress, due to the influence of external and internal factors (stressors), the internal balance – homeostasis – is disturbed (Barreto & Volpato 2004, Reeder & Kramer 2005). Stressors can be physical, psychological or combined in nature. Internal body stressors include e.g., hypoglycemia, anoxia; external ones include e.g., cold, heat, movement (work) activity or injury. Psychological stressors include stimuli that affect emotions (cause fear, anger, anxiety). In response to the stressor, a stress response occurs, which is a set of physiological and behavioural reactions and serves to neutralize the impact of the stressor and restore homeostasis (Reeder & Kramer 2005). Since it is necessary to mobilize as much energy as possible in these situations, there is an increase in the activity of the neural-autonomic system and humoral secretion (Barreto & Volpato 2004, Reeder & Kramer 2005, Renwanz & Spielvogel 2011). The most important physiological responses to stress in mammals stimulate the sympathetic nervous system and the HPA axis.

The first system ensures a very quick response – after activation of the sympathetic nervous system catecholamines adrenaline and noradrenaline are released. An increase in their levels immediately causes an increase in breathing rate, heart rate and blood pressure (Rietmann et al. 2004, Reeder & Kramer 2005, Renwanz & Spielvogel 2011). The second system is slower – after activation of the paraventricular nucleus of the hypothalamus, neurons begin to synthesize corticotropin (“releasing” hormone) and vasopressin. Corticotropin travels to the adenohypophysis and adrenocorticotropin (hereinafter referred to as ACTH) is released into the bloodstream. ACTH then acts on the adrenal cortex. Its influence results in the secretion of glucocorticoids, which increase available energy through increased glyconeogenesis, reduced glucose utilization, and reduced sensitivity to insulin (Reeder & Kramer 2005).

As indicated above, there is an increase in respiratory rate in response to stress. Respiratory frequency is thus often used in research as an indicator of stress (e.g., in fish, Barreto et al. 2003, Barreto & Volpato 2004, Bell et al. 2010, in birds, Carere & Oers 2004, Oers & Carere 2007, Davit et al. 2011, and in mammals Jürgens et al. 1996). However, a reaction to a stressful

situation can be quite individual (Bell et al. 2010). Intraspecific differences exist primarily in the rate of increase in the level of catecholamines (Koolhaas et al. 1999), and it can be assumed that individuals more easily subject to stress will breathe faster (Carere & Oers 2004, Bell et al. 2010, David et al. 2012), e.g., three-spined sticklebacks (*Gasterosteus aculeatus* Linnaeus, 1758) from populations with high predation pressure showed greater changes in respiratory rate than individuals from low-predation populations (Bell et al. 2010). In the case of “fast” and “slow” selected lines of great tits (*Parus major* Linnaeus, 1758), a striking relationship between the change in breathing rate and the personality type of individuals was found. Slow individuals had a higher breathing rate than fast individuals in both measurements at the beginning and at the end of the test (Carere & Oers 2004).

In our laboratory, we demonstrated the presence of consistent intraspecific behavioural variations (personality traits) in the common vole (Lantová et al. 2011, Urbánková et al. 2020), but have not yet tested the link to physiological parameters. From the above overview, this test would be beneficial and offer complementary information on how animals experience various behavioral tests and how it relates to personality traits. The following questions were answered:

- (1) Does the common vole show intraspecific variation in resting and stress breathing rate?
- (2) Are individual differences in breathing rate consistent over time?
- (3) Is intraspecific variation of breathing rate related to intraspecific variation of a behavioural trait?

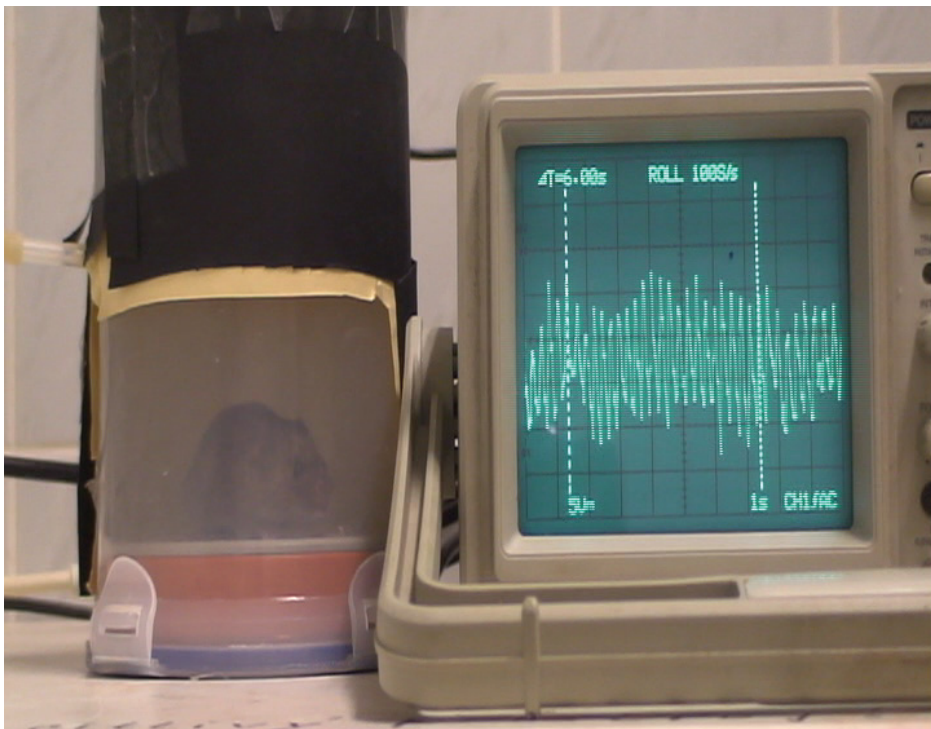


Fig. 1. Recording of the first measurement for vole ID 134; 21 breaths were detected in the video recording during a six-second period; multiplying the respiratory frequency by 10 gives $SBR=210 \text{ breaths} \times \text{min}^{-1}$.

MATERIAL AND METHODS

Vole individuals

The common voles, *Microtus arvalis* (Pallas, 1778), originating from the first generation of young individuals caught in the wild (České Budějovice, 48.9778° N, 14.4414° E) were used for the tests. In total, there were 30 individuals (11 males and 19 females) aged about 12 weeks. Voles were kept individually in polycarbonate breeding boxes 58×36×20 cm (VELAZ Praha) with wood shavings, hay, and a plastic tube as a shelter (l=15 cm, d=4 cm). Commercial pellets for rats and mice, as well as pellets for guinea pigs and rabbits (VELAZ Praha), fresh carrots, and water were available *ad libitum*. All individuals were individually marked on the breeding boxes. The laboratory conditions were stable, with a room temperature of 18–20 °C under a L:D 12:12 photoperiod, the same conditions as for their parents.

Breathing rate measurement

In small terrestrial mammals, measuring the breathing rate is quite difficult. In a study by Jürgens et al. (1996), the breathing rate of the pygmy white-toothed shrew, *Suncus etruscus* (Savi, 1822) was measured using electrocardiography (ECG) and verified using an auto-focusing laser system that measured chest movements. Another possibility is the use of a piezoelectric transducer, which can be combined with an ECG and thus a record of breathing and heart rate can be obtained simultaneously (Zehendner et al. 2013). In our study, the breathing rate was measured using a respirometric chamber with a volume of 3 dm³ connected to an electronic pressure transducer recording pressure changes in the chamber. Changes in electrical voltage from the transducer were displayed on the oscilloscope screen (Fig. 1). The chamber was equipped with a double bottom, into which was inserted a filter paper soaked in a solution of potassium hydroxide (concentration of 1 mol×l⁻¹), which served as a carbon dioxide absorber. The concentration of oxygen in the air is also important, a drop in concentration should not be more than 4% (see e.g., Nilsson 2010). We relied on data that a small mammal the size of a vole consumes 3 ml of oxygen per 1g of body weight per hour. Our experiment did not exceed the stated critical limit by far.

The tested vole was moved from its breeding box to the respirometric chamber using a plastic tube (home shelter). Breathing rate was measured for a total of 20 minutes and was recorded by a video camera. The tested individual was startled by a sound signal immediately after being placed in the chamber to obtain an individual stress breathing rate (SBR) value. After calming down (falling asleep, lying down), the resting breathing rate (RBR) was read. The latency of this behaviour as well as urination was also recorded. Each individual was weighed before and after all tests. After each animal, the chamber was washed with diluted alcohol and water then dried. Each vole was tested a total of three times (BM – breathing measurement 1–3). The interval between the first and second measurement was 24 hours, the third measurement was performed one month after the second. Output parameters (SBR, RBR, sedation latency) were evaluated from the video recording of each measurement, see Fig. 1. On the video recording, 21 breaths were recognized in a period of six seconds, by multiplying by 10, a respiratory rate of 210 breaths×min⁻¹ was obtained.

Personality trait

Personality trait was determined using open field tests (OFT; Hall 1934, Archer 1973). Restricted amount of data were used from the study Urbánková et al. (2021) where 5 tests were carried out with two-month intervals. The tested voles were placed in an opaque apparatus with dimensions of 75×75 cm, which was uniformly illuminated by an 18 W fluorescent lamp. The animals were transferred from the breeding box to the experimental apparatus in a plastic tube, which served as a shelter in the breeding box. The OFT took place for three minutes and was recorded by a digital camera (Panasonic Color CCTV Camera WV-CP500/G) placed 150 cm above the testing arena. Analysis of the video recordings was carried out using the “Modular tracking system 1.07” program, which was able to track the movement of the animal in the test apparatus. The voles were tested between 9 a.m. and 4 p.m. in a random order, because voles are animals with a polyphasic circadian activity with alternating 2–3 hours of moving and rest (Gerkema et al. 1993). After the end of each test, the apparatus was wiped with diluted alcohol and water, then dried with a cellulose cloth. The output information was the length of the trajectory given in meters. For organizational reasons, it was possible to take data from 13 tested animals (4 males and 9 females) – see Table 3.

Statistical analyses

To gain insight into the parameters we were working with, principal component analysis (PCA) implemented in CANOCO software was used to show their relationships (ter Braak & Šmilauer 2012). For determination of the personality trait, we used the original values of the distance moved in three OFT using the lmer function in R 4.0.2 (R Core Team 2020) using lme4 package. These analyses were done to generate the random intercepts (personality trait) for each individual (Urbánková et al. 2021). We used each OF test round (1–5) as an independent variable, ID for random effect and assessed the consistency repeatability (R_c) after Biro & Stamps (2015). We used an updated version of rptR package (Stoffel et al. 2017) enabling us to compute repeatability according to the distribution of each dependent variable, to assess intra-individual variation.

The same statistical procedure (LMM) was used for evaluation of the personality trait in the breathing rate categories and in the evaluation of the significance of the independent variables for the response variables of the three breathing

Table 1. Overview of the measured parameters

parameters	mean	median	minimum	maximum	SD
RBR (min^{-1})	141.5	140.0	100.0	210.0	22.1
SBR (min^{-1})	218.4	210.0	170.0	290.0	28.3
increase (%)	34.8	37.0	0.0	59.3	11.7
weight 1 (g)	26.5	23.3	15.1	50.0	9.0
weight 2 (g)	26.0	22.9	15.4	48.7	8.7

RBR (min^{-1}) – resting breathing rate, SBR (min^{-1}) – stress breathing rate, increase (%) – calculated from resting (100%) and stress values, weight 1 (g) and weight 2 (g) – weight before and after breathing measurement

rate categories. Figures were created in Statistica 13 (TIBCO Software Inc. 2017). Except for the relative change in breathing rate, no parameter had a normal distribution, so the parameters were logarithmically transformed. For all tests, we considered the cut-off for statistical significance as $P < 0.05$.

RESULTS

The measured values are presented in Table 1. The values for RBR ranged from 100 to 210 min^{-1} with mean value 141.5 min^{-1} and median 140 min^{-1} . The values for SBR ranged from 170 to 290 min^{-1} . The percentage values of the stress increase ranged from 0.0 to 59.3%, the mean value

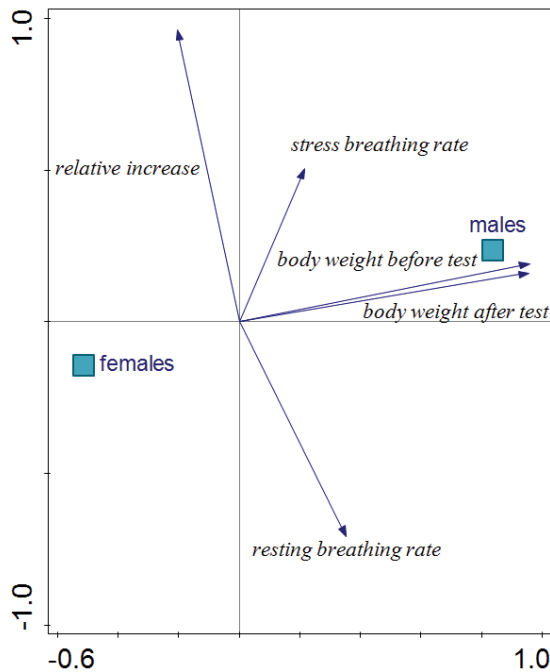


Fig. 2. Ordination graph of PCA for the measured parameters. Explained cumulative variations according to the axes 1, 2, and 3 were 41.4, 76.7, and 99.2%, respectively.

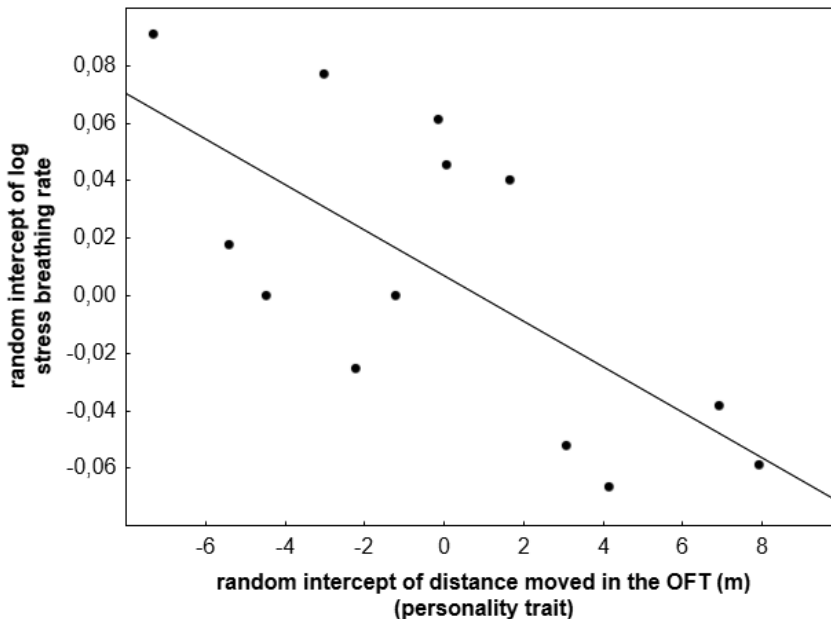


Fig. 3. Correlation between the random intercept of distance covered during the OFT and the stress breathing rate during the stress test ($r=-0.7069$, $p=0.0069$). The longer distance voles cover, the lower stress relative breathing rate increase is caused under stressful situations.

was 34.8 and the median 37.0. Body weights before the test ranged from 15.1 to 50.0 g with a mean value of 26.5 g and median 23.3 g. Body weights after the tests ranged from 15.4 to 48.7 g with mean value 26.0 g and median 22.9 g.

Using the PCA we got an overview of the relationships between the measured parameters. The relative increase in breathing rate during a stressful situation showed the opposite trend to resting breathing rate (Fig. 2). The lower the resting rate, the higher the stress increase. Sex factor along the first axis had a negligible effect on the mentioned physiological parameters. The body weights of the animals before and after the experiment showed an almost identical course, i. e. the test had small effect on the animals. Both weight vectors showed weak correlation with breathing parameters. The influence of sex and body weight was verified also by LMMs.

Based on LMM of the physiological parameters (RBR, SBR, and relative breathing rate increase), we tested for the significance of the fixed and random effects, shown in Table 2, the random intercepts are sorted in Figs. S1–3. In Table 3, this high variation of individuals is characterised by consistency and repeatability $R_c=0.58$, 0.77 , and 0.60 , respectively. Besides the measured parameters, we also included distance moved in OFT in the evaluations, taken with the author's kind permission (Urbánková et al. 2021) from the data pool of the personality trait stability study (Tables 2 and 3). Unfortunately, we could use data for 13 voles only. Their IDs are listed in Table 3. Random intercepts of distance moved in OFT were considered as certain personality traits and correlations with the physiological parameters were determined (Table 3). A statistically insignificant value $r=0.1414$ was found for the resting breathing rate. Statistically significant correlation was determined in the correlation of personality trait and the random intercept of stress breathing

Table 2. Significance of the fixed and random effects in LMM for four response variables. Presented tests compare each model with the reduced model on the preceding row

response variable	model	AIC	BIC	log lik	df	p
distance	~ trial	456.30	462.82	-225.15		
	~ trial + (1 ID)	443.22	451.92	-217.61	1	0.0001
	~ trial + sex + (1 ID)	445.11	455.98	-217.55	1	0.734
	~ trial + weight + (1 ID)	444.37	455.25	-217.19	1	0.357
log RBR	~ trial	-216.25	-208.86	111.13		
	~ trial + (1 ID)	-241.30	-231.44	124.65	1	<0.000
	~ trial + sex + (1 ID)	-239.42	-227.09	124.71	1	0.736
	~ trial + weight + (1 ID)	-240.12	-227.79	125.06	1	0.367
log SBR	~ trial	-254.52	-247.12	130.26		
	~ trial + (1 ID)	-310.36	-300.49	159.18	1	<0.000
	~ trial + sex + (1 ID)	-308.98	-296.65	159.49	1	0.428
	~ trial + weight + (1 ID)	-311.46	-299.13	160.73	1	0.078
relative increase	~ trial	-122.42	-115.02	64.21		
	~ trial + (1 ID)	-149.47	-139.61	78.74	1	<0.000
	~ trial + sex + (1 ID)	-147.51	-135.18	78.75	1	0.853
	~ trial + weight + (1 ID)	-147.48	-135.15	78.74	1	0.920

AIC – Akaike information criterion; BIC – Bayesian information criterion; log lik – log-likelihood *estimates*; df – degrees of freedom; p – significance level.

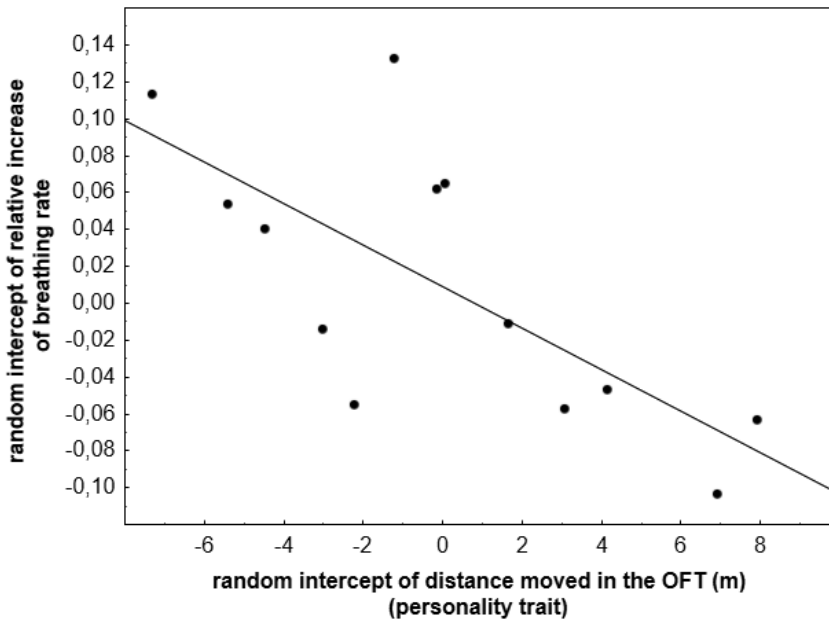


Fig. 4. Correlation between the random intercept of distance covered during the OFT and the relative increase of stress breathing rate during the stress test ($r=-0.7069$, $p=0.0069$). The longer distance a vole covers, the lower relative breathing rate increase is caused under stressful situations.

Table 3. Intercepts of random effects of LMM in one behavioural parameter considered personality trait and three physiological parameters

ID	sex	distance personality	log RBR	log SBR	breathing increase
5	+ + + + + + + + + + + + + +	-1.2188	-0.0957	-0.0001	0.1324
47		6.9250	0.0315	-0.0384	-0.1039
60		-2.2169	0.0160	-0.0252	-0.0550
64		-3.0250	0.0816	0.0771	-0.0142
71		1.6490	0.0466	0.0404	-0.0115
72		-7.3028	-0.0014	0.0911	0.1134
75		0.0804	-0.0120	0.0455	0.0645
76		-4.4826	-0.0261	-0.0001	0.0401
77		3.0749	-0.0082	-0.0523	-0.0577
81		4.1523	-0.0273	-0.0665	-0.0468
88		-0.1572	0.0160	0.0614	0.0618
90		-5.4174	-0.0180	0.0175	0.0537
92		7.9390	0.0154	-0.0590	-0.0631
R±SE			0.43±0.14	0.58±0.10	0.77±0.07
p		<0.0001	<0.0001	<0.0001	<0.0001
r			0.1414	-0.6889	-0.7069
p			0.6450	0.0092	0.0069

RBR – resting breathing rate, SBR – stress breathing rate, log – logarithm of base 10, R – repeatability, SE – standard error, r – correlation coefficient, p – significance level, bold – significant value.

rate ($r=-0.689$, $p=0.009$, Fig. 3). The voles with a higher breathing rate under stressful conditions show a shorter movement trajectory in the OFT. Higher correlation was determined with the relative stress increase ($r=-0.707$, $p=0.007$, Fig. 4). This shows that the longer the distance a vole covers in the OFT, the lower relative breathing rate increase is caused under stressful situations.

DISCUSSION

Determined values and stress increase of breathing rates

The values for RBR ranged from 100 to 210 min^{-1} with mean value 141.5 min^{-1} and median 140 min^{-1} . The determined values correspond with other published data. Breathing rate corresponds with metabolic rate and in mammals also to the weight of the animals (Crosfill & Widdicombe 1961). In mammals such as the cat, the respiratory rate ranges from 20 to 50 min^{-1} and in the dog from 10 to 40 min^{-1} (Kleinman & Radford 1964). The breathing rate of smaller mammals, such as mice, ranges from 80 to 200 min^{-1} , in hamsters and rats from 60 to 110 min^{-1} , in guinea pigs from 70 to 100 min^{-1} (Kleinman & Radford 1964) and laboratory mice about 200 min^{-1} (Sahai et al. 2021). In the pygmy white-toothed shrew, the average respiratory rate is around 661 min^{-1} (Jürgens et al. 1996). For the cinereus shrew (*Sorex cinereus* Kerr, 1792) weighing 3.4 g, it is 800 min^{-1} (Morrison et al. 1953 ex Calder 1968).

The values for SBR ranged from 170 to 290 min^{-1} . The percentage values of the stress increase ranged from 100% to 171%, whereas the mean value was 134.8% and the median 137.0%. The determined values correspond to the published data and approximately mirror common stress intensity. In mice, the mean breathing rate increased from 180 to 246 (137%) after a drug (acetazolamide) was injected (Sato et al. 2018). In injured rats, the mean values increased from about 110 min^{-1} to 230 min^{-1} (209%; Baran et al. 2020).

In mice, a changed air composition increased the breathing rate by about 150 % to over 200 % from basic breathing rate of about 150 min⁻¹ (Receno et al. 2019). Tube restraint in mice changed the breathing rate from 220 to 300 min⁻¹ (136%) and after bicuculline (plant alkaloid) injection from 250 to 380 min⁻¹ (152%; Li et al. 2020).

Intraspecific variation and consistent individual differences

In the common vole, intraspecific variation of the breathing rate was found. Variation was found in all parameters, in resting breathing rate, stress breathing rate, and stress breathing relative increase. Study of intra- and inter-individual variations and their consistency of behavioral and physiological parameters, especially in mammals, has been given attention for a long time (Hayes & Jenkins 1997). As can be seen from the previous chapter, the observed intraspecific variation corresponds to that reported in the literature. The main sources of this variation could be caused by differences in sex and weight of the individuals. However, none of the mentioned factors had a significant influence on the observed breathing rate parameters (see Fig. 2 and Table 2). Although the breathing rate is a physiological parameters that contribute to maintaining the narrow range of homeostasis of the internal environment, under natural conditions, heterozygotes tend to be favored, especially for polymorphisms in natural populations (Parsons 1998). This finding is consistent with studies that have also found intraspecific variation in breathing rate in birds e.g., in great tit (Carere & van Oers 2004, Fučíková et al. 2009) or in zebra finch (David et al. 2012). The variation was the same or similar to that observed in the case of other physiological parameters (overview in Koolhaas et al. 1999).

We found that the resting breathing rate ($R_c=0.58$), and stress breathing rate ($R_c=0.77$), as well as, the percentage stress increase ($R_c=0.60$) have a high degree of repeatability at the level of the individual. This indicates that the observed intraspecific differences/variation are not the result of imprecise measurement or changing laboratory conditions, but rather a manifestation of the individual characteristics of the tested voles. This result is consistent with other studies demonstrating the consistent nature of individual differences in breathing rate values. A study on zebra finches reported high repeatability ($R=0.66$) in individual respiratory rate values (David et al. 2012). The repeatability of respiratory rate values in individuals may be related to the length of interval between individual measurements and their timing in the ontogeny of the monitored individuals, as follows from the study by Fučíková et al. (2009). There, the repeatability of the stress breathing rate in the great tit was measured, first at the age of 14 days and again at six months of age. The detected value ($R=0.34$) is about half compared to the values discussed above. However, as already mentioned, this difference is most likely caused by different experimental design. In our study and the study by David et al. (2012) the breathing rate of adult animals was measured. Fučíková et al. (2009) measured baseline values relatively shortly after hatching, and these can change significantly during maturation and reduce the repeatability value. For other physiological indicators of stress, similarly high values can be found in other studies. For example, the repeatability values of stress heart rate and corticosterone level at the individual level in the goose were $R=0.84$ and 0.77 , respectively (Kralj-Fišer et al. 2007, 2010). For glucocorticoid metabolites, high repeatability was found also in rats ($R=0.51$) (Vobrubová et al. 2021). This is understandable because these parameters are related to the internal environment where high stability is required to maintain homeostasis. However, the repeatability of physiological parameters strongly depends on intervals between individual measurements and their duration to ensure stability (Ferrari et al. 2013, Karlíková et al. 2018). With behavioural expressions of stress responses, the situation is somewhat different, here the general repeatability is approximately 37% (Bell et al. 2009). In voles, depending on the specific behavioural parameter, the repeatability ranged between 20% to 63% (Lantová et al 2011, Herde & Eccard 2013, Urbánková et al. 2020).

Correlation of physiological and behavioural variation

Resting breathing rate (RBR) represented by random intercept (personality trait) was not related to the random intercept (personality trait) of the trajectory length traveled by the animal in the Open Field tests. The rationale may be that intraspecific differences in behaviour are most pronounced in stressful contexts (Koolhaas et al. 1994). In our case, this is in the OFT, where the animals are not in a resting state. At the inter-population level, this finding can also be supported by the results of a study conducted on the three-spined stickleback, where no differences were found in the basal breathing rate in populations living in environments with different predation pressure (Bell et al. 2010). Stress breathing rate (SBR) represented again by random intercept was negatively related to the random intercept of length of the trajectory traveled by the vole in the OFT. A similar trend was found in the percentage increase from RBR to SBR. Voles that reacted to the induced stress (manipulation and acoustic startle) with a greater increase in breathing rate displayed behaviour as anxious (“shy” personality type) in OFT. Their locomotor activity was reduced by the stress induced during exposure in the OFT. The obtained results point to the fact that stress seems to induce a more intense physiological response in individuals of the shy behavioural type, which in our study was manifested by a rapid increase in breathing frequency. We can find similar reactions in great tits, where breathing rate was significantly higher in “slow” individuals (analogy of the shy personality type) than in “fast” (“bold”) birds (Carere et al. 2001, Carere & van Oers 2004). However, as already indicated in the introduction, the results of studies carried out so far focusing on the relationship between breathing rate and personality traits do not completely match. One such study is on zebra finches (David et al. 2012). The authors experimentally investigated to what extent handling stress was correlated with personality in female zebra finches, as assessed from a suite of repeatable behavioural traits. Although breathing rate was repeatable across individuals, it was not related to any behavioural trait, suggesting that it cannot be used to quickly predict personality, at least in zebra finches. The following study fits well into this seemingly contradictory picture too.

Fučíková et al. (2009) showed a completely opposite relationship, where fast great tits showed a higher stress breathing rate compared to slow individuals. This corresponds to Koolhaas et al. (1999) that formulated a more general view that proactive coping rodents show, in response to stressful stimulation, a low HPA-axis reactivity (low plasma corticosterone response), but high sympathetic reactivity (high levels of catecholamines). In contrast, reactive coping rodents show higher HPA axis reactivity and higher parasympathetic reactivity. However, it should be also noted that the activity of the sympathetic nervous system in general and the level of plasma noradrenaline, in particular, are connected with the metabolic and cardiovascular demands associated with physical activity, which could explain the positive correlation between proactive coping and sympathetic reactivity (Koolhaas et al. 2010, Kanitz et al. 2019). A similar correlation was also observed in the bank vole. Bolder and less flexible individuals had lower faecal glucocorticoid metabolite levels than shy, more flexible individuals (see Mazza et al. 2019). This inconsistency may be caused, as already mentioned above, by a different experimental design. In our study common voles could mirror an emotional stress and not a physical one, because they were held in a small respiration box and soon after insertion displayed quite limited locomotor activity. It could therefore be thought that the original coping style concept effectively needs replacement for a new coping style model with two axes (Mazza et al 2019).

Despite the correlation of breathing rate with a personality trait was relatively high, a generalisation is highly restricted due to the low number of evaluated individuals. Besides correlation with higher number of behaviourally tested animals, also data on faeces pellets and urination produced during all tests should be involved in the analysis. However not only the quantity, but also the precise timing of voiding. The precise knowledge of timing is important also for the breathing

rate increase because it is important to distinguish the fast response mediated above all by neural pathways and the reaction mediated by above all hormonal substances. These differences could be associated with personality traits as was mentioned above. The finding that breathing rate could indicate a personality trait would be very valuable, not only from the point of view of behavioural ecology, but also for other aspects. Undisputed advantages include the time-saving way in which individual values of this parameter could be obtained, but above all its non-invasiveness. This is beneficial not only from an ethical point of view, but also from a methodological point of view. So far, the only method that approximates the measurement of breathing rate with its advantages is determining the values of stress hormones from excrement (Vobrubová et al. 2021). In this case, however, it is always difficult to compare the results between individuals with regard to the difference in behaviour over a longer period of time, for which the faeces are analysed.

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SUPPLEMENT

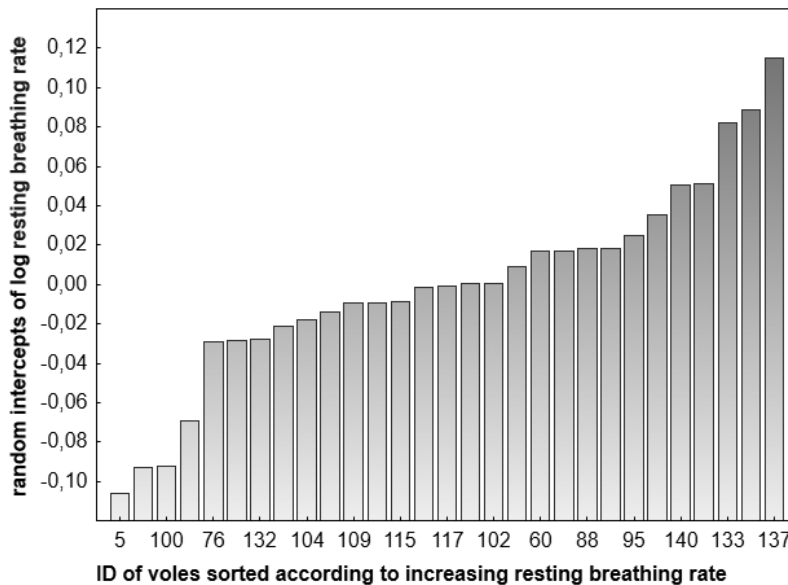


Fig. S1. Resting breathing rate (RBR, min^{-1}) was log-transformed and evaluated by linear mixed model (LMM). The bars show random intercepts that represent specific individuality traits of the measured physiological parameter. The figures are the IDs of the tested voles, $n=29$.

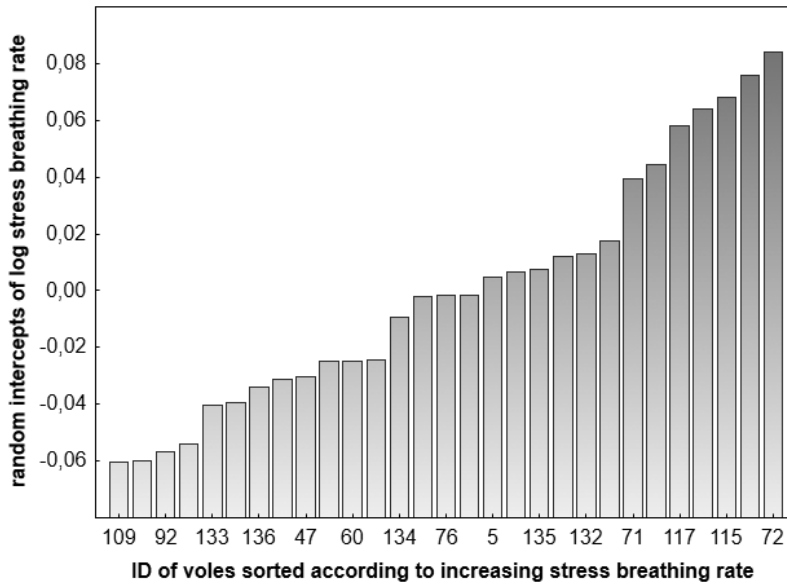


Fig. S2. Stress breathing rate (SBR, min^{-1}) was log-transformed and evaluated by linear mixed model (LMM). The bars show random intercepts that represent specific individuality traits of the measured physiological parameter. The figures are the IDs of the tested voles, $n=29$.

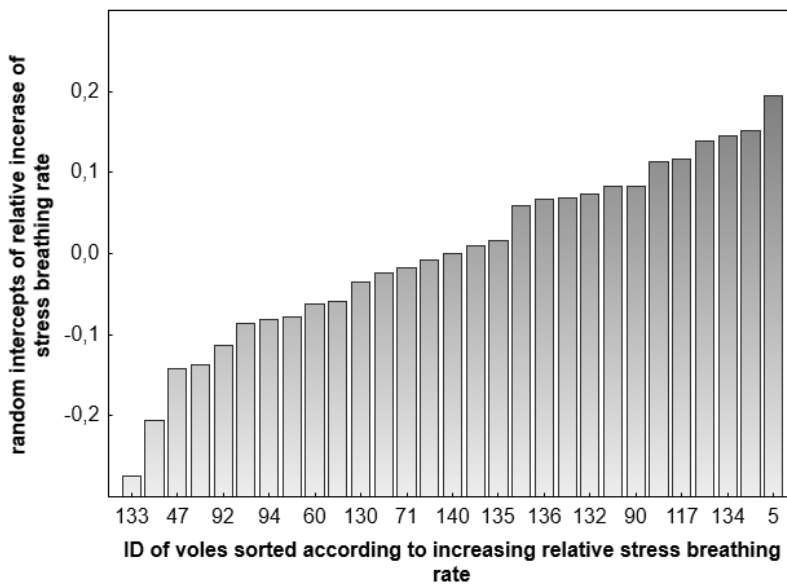


Fig. S3. Relative increase of stress breathing rate was evaluated by linear mixed model (LMM). The bars show random intercepts that represent specific individuality traits of the measured physiological parameter. The figures are the IDs of the tested voles, $n=29$.

Scorpions of the Horn of Africa (Arachnida: Scorpiones). Part XXIX. A new species of *Neobuthus* from Somaliland (Buthidae)

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Abstract. A new species *Neobuthus fryntai* sp. nov. confirmed by DNA phylogeny (paper in preparation) is described according to morphological characters. It is morphologically most similar to *N. factorio* Kovařík, Lowe, Elmi et Hure, 2018. *Neobuthus fryntai* sp. nov. has pedipalp segments relatively slender than *N. factorio*, males with femur L/W 2.74–2.86 (2.42–2.70 in *N. factorio*), patella L/W 2.50–2.62 (2.18–2.28 in *N. factorio*), chela L/W 4.68–4.98 (4.25–4.48 in *N. factorio*). Included is actualized distribution map of the genus *Neobuthus* Hirst, 1911 with focus to Somaliland.

Key words. Taxonomy, new species, Scorpiones, Buthidae, *Neobuthus*, Somaliland, Afrotropical region.

INTRODUCTION

The genus *Neobuthus* Hirst, 1911 so far includes 16 small buthid scorpions distributed in northeastern Africa (Horn of Africa). The genus has been revised in Kovařík et al. (2018) and Kovařík (2019). However, the history of the genus was confusing until Kovařík & Lowe (2012) redefined its taxonomic concept and clarified its differences from the genus *Butheolus* Simon, 1882. In 2020–2022 continued the study of scorpion's fauna in Somaliland with cooperation of Charles University in Prague (Czech Republic) and Amoud University in Borama (Republic of Somaliland). This study produced another new species of the genus *Neobuthus* described here and new data about distributions added in the map with distribution of genus *Neobuthus* (Figs. 44–45).

MATERIAL AND METHODS

Nomenclature and measurements follow Vachon (1963), Stahnke (1971), Sissom (1990), Kovařík (2009), and Kovařík & Ojanguren Affilastro (2013), except for trichobothriotaxy (Vachon 1974, 1975), and morphology of sternum (Soleglad & Fet 2003).

Specimens used for this study were collected and imported with permissions from Amoud University and Ministry of the Environment of the Republic of Somaliland.

Specimens studied herein are preserved in 80% ethanol in the first author collection (FKCP, František Kovařík, private collection, Prague, Czech Republic; will in future be merged with the collections of the National Museum (Natural History), Prague, Czech Republic). Map documents for Figs. 44–45 were downloaded at <https://www.simplenamap.net/#tabs=1>.

TAXONOMY

Buthidae C. L. Koch, 1837

***Neobuthus* Hirst, 1911**

(Figs. 1–70, Table 1)

Neobuthus Hirst, 1911: 462; Kovařík & Lowe 2012: 1–25, figs. 1–6, 9–74, 86, 89, 92, 95–96, 100–101 (including complete generic synonymy prior to 2012); Kovařík et al. 2013: 4, 14; Lowe & Kovařík 2016: 1–46, figs. 1–165, tables 1–5; Kovařík et al. 2018: 1–82, figs. 1–438, tables 1–5; Kovařík 2019: 1–16, figs. 1–70, table 1.

TYPE SPECIES. *Neobuthus berberensis* Hirst, 1911.

DIAGNOSIS. Small buthid scorpions, total length 15–25 mm (males), 22–32 mm (females); carapace strongly trapezoidal, surface granular with only anterior median carinae developed; ventral aspect of cheliceral fixed finger with single denticle; tergites with three carinae, of which the lateral pair may be less conspicuous; sternites III–VI with finely micro-denticulate posterior margins, lacking larger non-contiguous denticles; pectines with fulcra, hirsute; metasomal segments I–III with 8–10 carinae, segment V with enlarged lobate dentition on posterior ventrolateral carinae; telson rather bulbous, vesicle steeply inclined posteriorly, aculeus shorter than vesicle; macrosetae on vesicle normal to surface (♂) or oriented in anterior direction (♀); pedipalps short with stout segments, movable finger of pedipalp with 4–6 subrows of primary denticles flanked by mid-row internal and proximal external accessory denticles, 3 denticles just proximal to terminal denticle; movable finger without dense terminal brush of setae on ventral surface; strongly spatulate macrosetae not present on termini of fixed and movable fingers; pedipalp finger margins straight, without proximal scalloping or basal lobe and notch; trichobothrial pattern type A, orthobothriotaxic or neobothri-



Fig. 1. *Neobuthus fryntai* sp. nov., female paratype in vivo habitus.

Table 1. Comparative measurements of adults of *Neobuthus fryntai* sp. nov. Abbreviations: length (L), width (W, in carapace it corresponds to posterior width), depth (D)

dimensions (MM)		<i>Neobuthus fryntai</i> sp. nov. ♂ holotype (347)	<i>Neobuthus fryntai</i> sp. nov. ♀ paratype (356)
carapace	L / W	2.90 / 3.11	3.44 / 4.05
mesosoma	L	6.90	7.46
tergite VII	L / W	1.67 / 3.10	1.76 / 3.93
metasoma + telson	L	16.09	17.12
segment I	L / W / D	2.11 / 1.82 / 1.55	2.11 / 2.44 / 2.10
segment II	L / W / D	2.22 / 1.69 / 1.66	2.49 / 2.22 / 2.07
segment III	L / W / D	2.46 / 1.63 / 1.67	2.60 / 2.18 / 2.09
segment IV	L / W / D	2.81 / 1.57 / 1.44	3.03 / 2.04 / 1.94
segment V	L / W / D	3.34 / 1.52 / 1.37	3.67 / 2.00 / 1.62
telson	L / W / D	3.15 / 0.99 / 0.94	3.22 / 1.38 / 1.24
pedipalp	L	8.53	8.56
femur	L / W	2.06 / 0.72	2.09 / 0.78
patella	L / W	2.78 / 1.06	2.75 / 1.18
chela	L	3.69	3.76
manus	W / D	0.74 / 0.72	0.87 / 0.90
movable finger	L	2.56	2.20
total	L	25.89	28.02

otaxic minorante, dorsal trichobothria of femur arranged in β -configuration; trichobothrium d_2 of pedipalp femur present or absent on dorsal surface, d_2 of pedipalp patella present or absent, d_3 of pedipalp patella situated internal to dorsomedian carina, V_2 of chela manus strongly displaced internally relative to V_1 , chela fixed finger with db located in proximal half, proximal to est ; tibial spurs present on legs III–IV; sexual dimorphism in setation, granulation and metasomal dentition: pedipalps, legs and metasoma with weaker granulation and long, filiform macrosetae in females, stronger granulation and shorter (often spiniform) macrosetae in males, ventrosubmedian and ventrolateral carinae on segments II–III strongly developed with enlarged dentition in females and regular dentition in males; capsule of hemispermatophore with 4 lobes in typical 3+1 configuration, basal lobe a broad, robust, hook-like process, flagellum well separated from lobes.

***Neobuthus fryntai* sp. nov.**

(Figs. 1–45, Table 1)

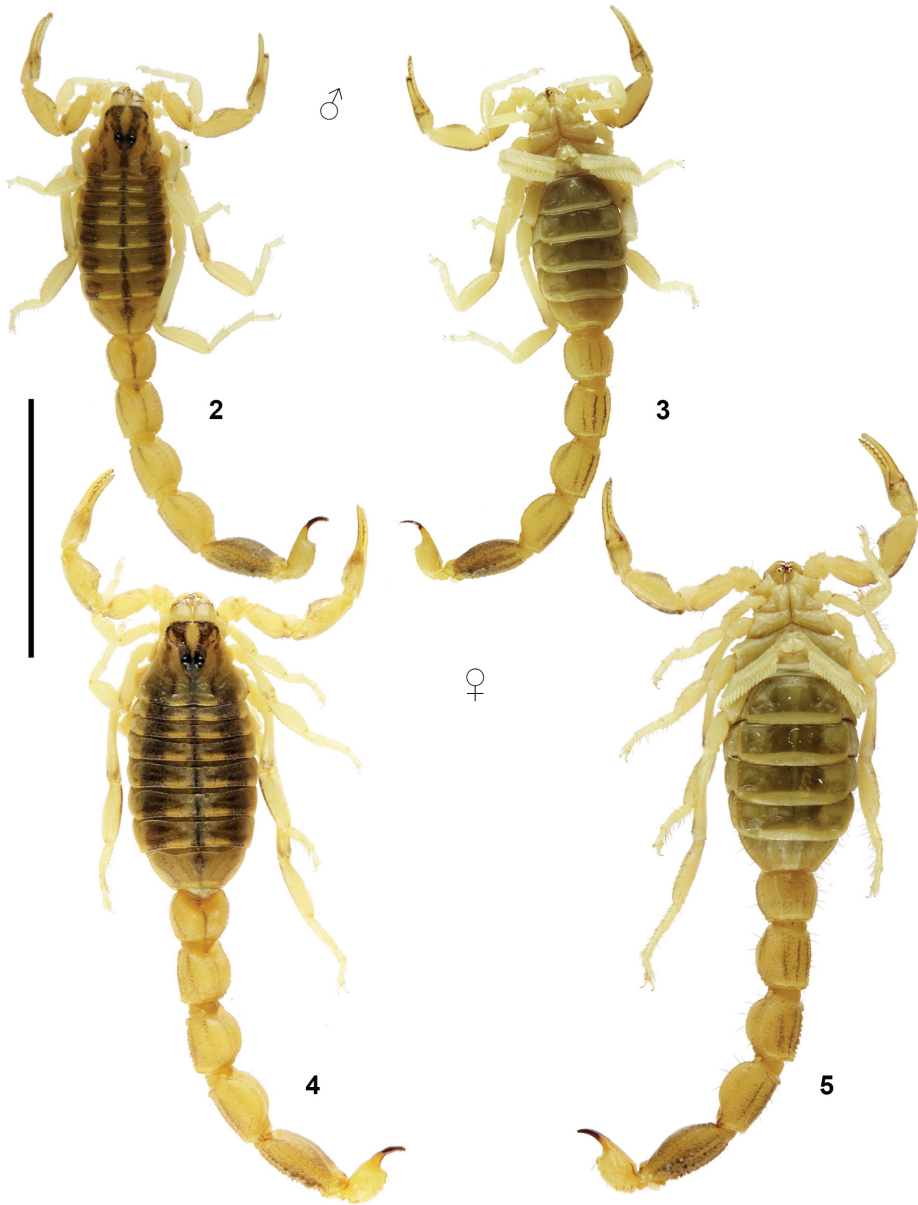
TYPE LOCALITY. Somaliland, Gacan Libaax Mts, Kalwarabe, 09°58'03"N, 45°03'11"E, ca. 1600 m a. s. l.

TYPE MATERIAL. **Somaliland**, Gacan Libaax Mts, Kalwarabe, 09°58'03"N, 45°03'11"E, ca. 1600 m a. s. l., 19–20 VI 2022, 8 ♂♂, 2 ♀♀, holotype, DNA No. 341 and paratypes, DNA Nos. 344, 347, 349, 352, 353, 356, 357, 361, 362), leg. Abdurahman Elmi et al.; FKCP.

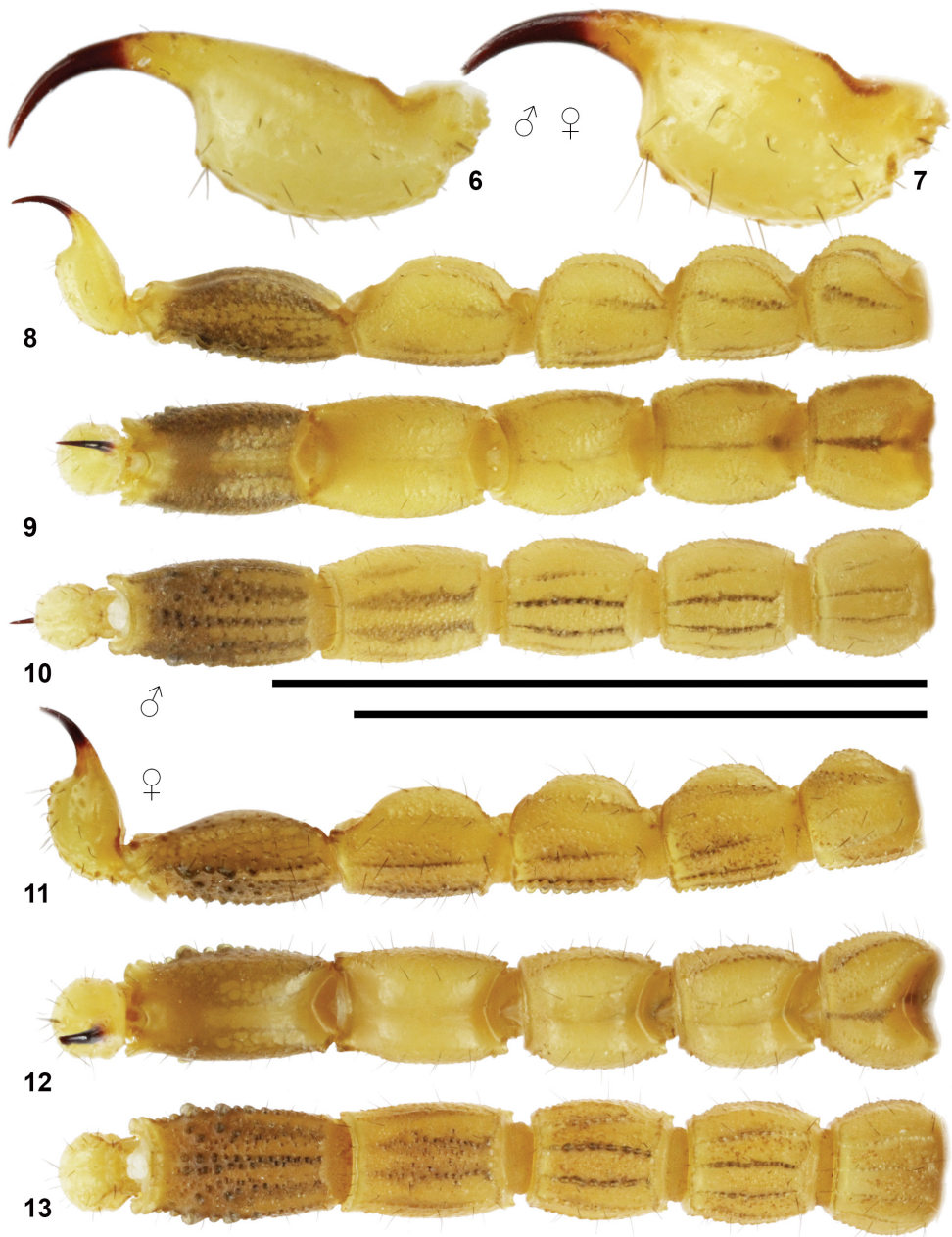
ETYMOLOGY. The specific epithet honors Daniel Frynta, a zoologist and professor from Charles University in Czech Republic and our friend, who established an extensive research of species diversity in the Republic of Somaliland in 2016. He visited Somaliland several times and established a successful collaboration with Amoud University in Borama.

DIAGNOSIS. Total length 22–26 mm (males), 24–29 mm; carapace with area between anterior median carinae yellow to orange; tergites with 3 dark stripes, median stripe flanked on either side

by broad longitudinal yellow bands that may be broken by fuscidity extending across anterior tergites; pedipalp relatively slender, males with femur L/W 2.74–2.86, patella L/W 2.50–2.62, chela L/W 4.68–4.98; chela movable finger with 6 subrows of primary denticles, 4–5 external



Figs. 2–5. *Neobuthus fryntai* sp. nov. 2–3 – Male paratype (No. 341), dorsal (2) and ventral (3) views. 4–5 – Female paratype (No. 356), dorsal (4) and ventral (5) views. Scale bar 10 mm.



Figs. 6–13. *Neobuthus fryntai* sp. nov. 6, 8–10 – Male paratype (No. 341), telson lateral (6), metasoma and telson lateral (8), dorsal (9), and ventral (10) views. 7, 11–13 – Female paratype (No. 356), telson lateral (7), metasoma and telson lateral (11), dorsal (12), and ventral (13) views. Scale bars 10 mm (8–10, 11–13).

accessory denticles flanking proximal end of each subrow; trichobothria d_2 usually present from femur and patella; dorsoexternal and ventroexternal carina on pedipalp patella in female weakly indicated to absent; smooth dorsal carinae on pedipalp chela present; posterior margins of tergites with 1–2 pairs of macrosetae; pedipalps, legs, metasoma and telson with moderately short, not spiniform macrosetae in males, and long, fine setae in females; males with sternites III–VI shagreened to smooth medially, sternite VII shagreened with 4, granulated carinae; females with sternites III–VI smooth, sternite VII with 4 weak granulated carinae; metasoma I–III with median lateral and dorsal carinae present in both sexes; lateral surface of metasoma V granulated in both sexes, with granules separated; soles of telotarsi with relatively sparse setation, leg III of adults with 9–13 ventral macrosetae on telotarsus; pectine teeth: 16–21 (males), 15–16 (females).

DESCRIPTION. Total length of adult males 22–26 mm, of adult females 24–29 mm; measurements of carapace, telson, segments of metasoma and pedipalps given in Table 1; positions and distribution of trichobothria of pedipalps shown in Figs. 15–18 and 20–21; trichobothrium d_2 usually present from femur and patella; base color pale yellow with variable fuscous pigmentation (Figs. 1–5) and patterns of dark maculation on metasoma, pedipalps and legs; chelicerae yellow with dark reticulation on anterior manus, dentition reddish. *Sexual dimorphism:* strong, adult males substantially smaller, but without differences in shapes of pedipalps, metasoma and telson; pedipalp patella and femur granulate and matte in males, smooth and glossy in females; sternites smooth in females and shagreened to smooth medially in males; macrosetae on pedipalps, legs, metasoma and telson much longer and finer in females than males; other sex differences cited below.

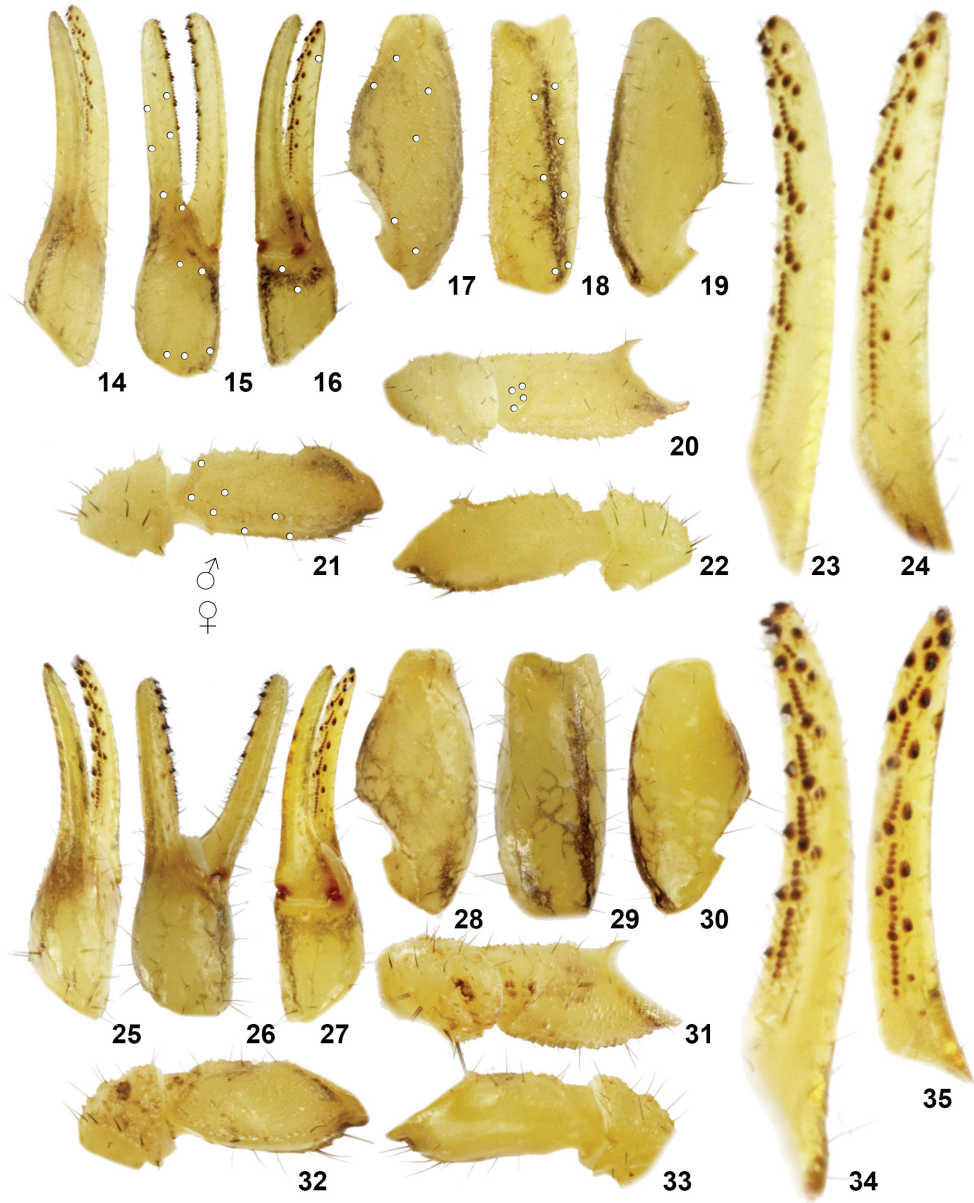
Pedipalp (Figs. 14–35). Pedipalp mostly sparsely hirsute; finely granulated in males and smooth except femur in females; femur with five conspicuously granulose carinae, more strongly developed in males; patella with seven granulose carinae, weakly developed in males and weakly indicated in females; chela with at least dorsal carinae present.

Carapace (Figs. 36, 38). Strongly trapezoidal (narrower anteriorly), wider than long (L/W 0.84–0.93); posterior median postocular area flat, anterior median preocular area gently sloped downwards towards anterior margin; lateral flanks steeply sloped; ocular tubercle broad, prominent, located slightly anterior to middle of carapace; anterior margin straight, finely microdenticulate, with coarser granules overlapping edge, bearing 8 macrosetae; anterior median carinae present, coarsely granular, other carinae indistinct; dense granulation covering most of carapace.

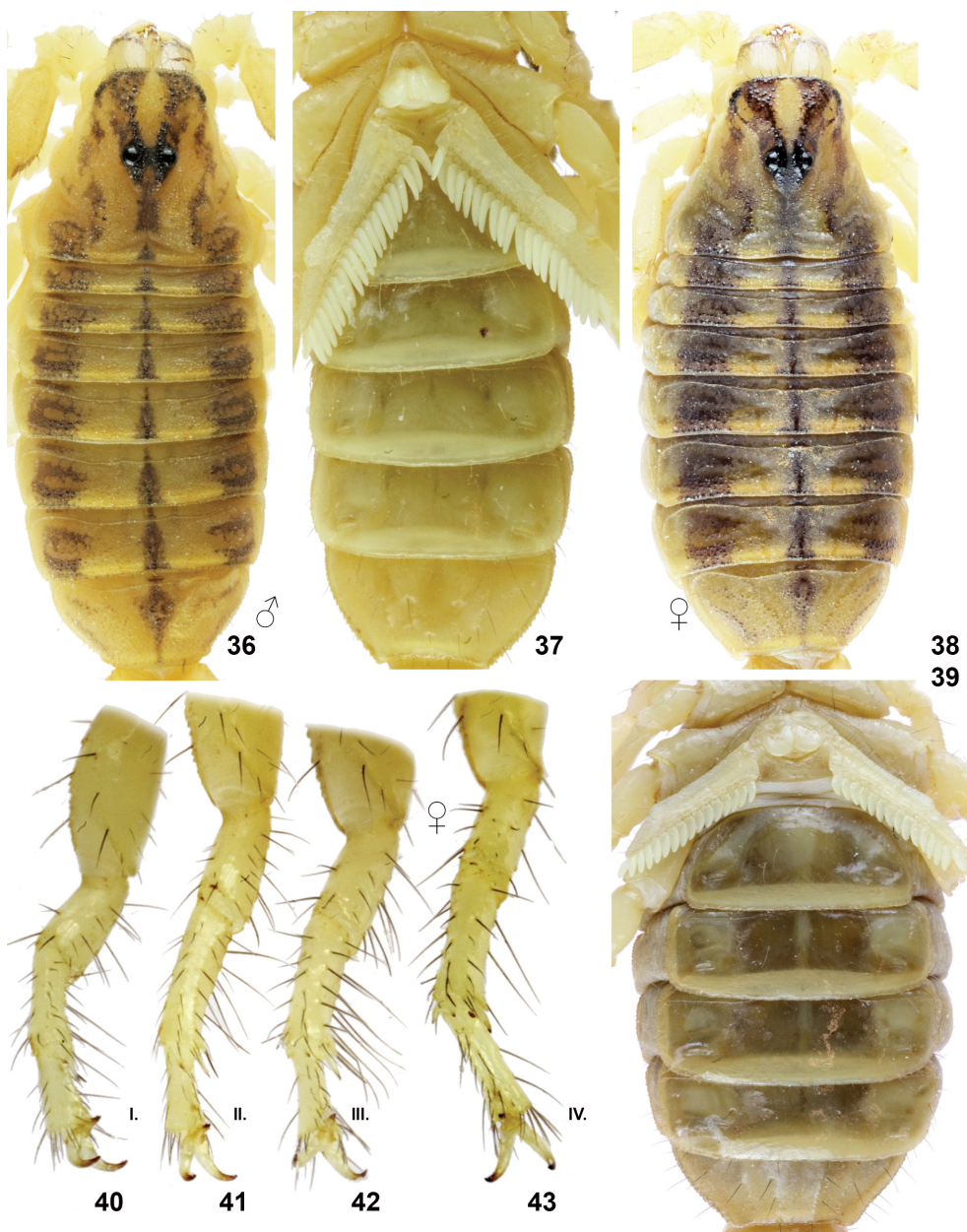
Chelicera (Figs. 36, 38). Fingers with typical buthid dentition (Vachon 1963, Lowe & Kovařík 2016); fixed finger with large distal denticle, 1 subdistal denticle and 2 basal denticles fused into bicuspid, single denticle on ventral surface at level of bicuspid; dorsal margin of movable finger with 5 denticles: 1 large distal denticle, medium-sized subdistal and medial, and 2 small, partially fused basal denticles; ventral margin with 2 denticles.

Mesosoma (Figs. 36–39). Tergites I–VI bear three carinae of which the lateral pair may be less conspicuous mainly on tergites I–IV; tergite VII bears five well-defined carinae (median, submedians and laterals); tergites I–VI densely granular, with coarser granules on posterior lateral areas; tergite VII densely granular; sternites III–VI smooth in females, and shagreened to smooth medially and granulate laterally in males; sternite VII granulated in both sexes, more so in males, with four weak to well-defined carinae; sternum type 1, triangular in shape; smooth, with deep posteromedian invagination; genital opercula smooth; genital papillae present; pectines extending to around end of sternite IV in male and around a quarter of sternite IV in female; pectine teeth 16–21 in males, 15–16 in females; combs with 3 marginal lamellae and 7–8 middle lamellae; marginal lamellae, middle lamellae and fulcra with dense cover of short dark reddish macrosetae; fulcra with 2–4 setae.

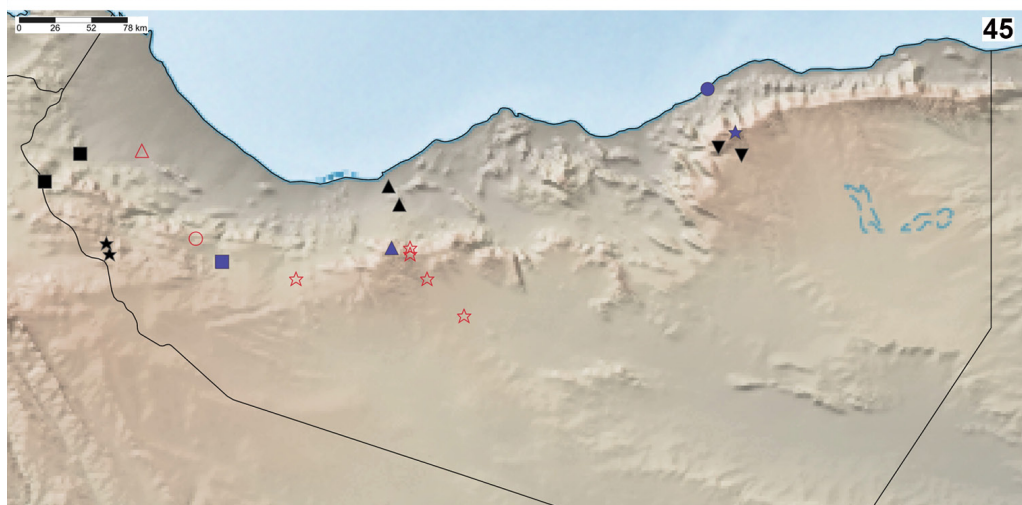
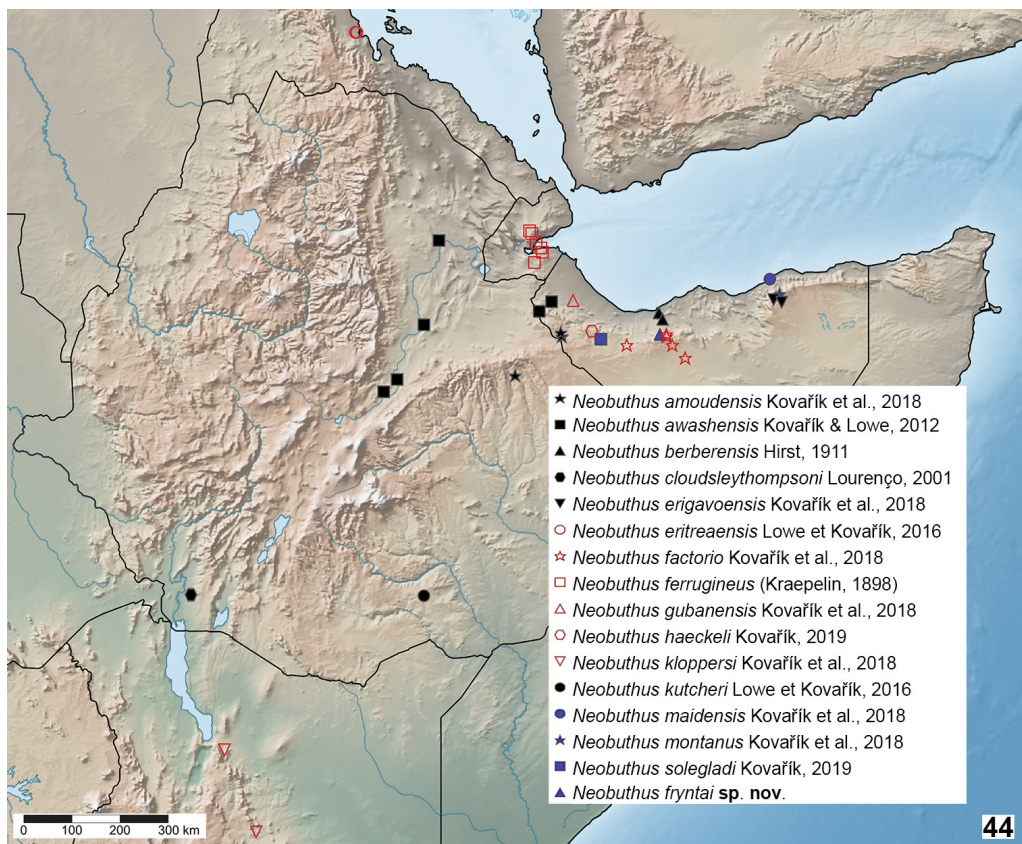
Legs (Figs. 40–43). Coxa, femora, patella and tibia of all legs bearing variable numbers of medium length, straight, dark-reddish macrosetae; tarsi with mix of short and longer, dark-reddish



Figs. 14–35. *Neobuthus fryntai* sp. nov., pedipalp. 14–24 – Male holotype (No. 347), chela dorsal (14), external (15), and ventral (16) views, patella dorsal (17), external (18) and ventral (19) views, femur and trochanter internal (20), dorsal (21), and ventral (22) views, movable (23) and fixed (24) finger dentate margin. 25–35 – Female paratype (No. 356), chela dorsal (25), external (26), and ventral (27) views, patella dorsal (28), external (29) and ventral (30) views, femur and trochanter internal (31), dorsal (32) and ventral (33) views, movable (34) and fixed (35) finger dentate margin. The trichobothrial pattern is indicated in Figs 15–18, 20–21 (white circles).



Figs. 36–43. *Neobuthus fryntai* sp. nov. 36–37 – Male holotype (No. 347), carapace and tergites (36), coxosternal area and sternites (37). 38–43 – Female paratype (No. 356), carapace and tergites (38), coxosternal area and sternites (39), and left legs I–IV, retrolateral aspect (40–43).



Figs. 44–45. Map showing confirmed distribution of *Neobuthus* spp. (44) with detail for Somaliland (45).

macrosetae; basitarsi I–III slightly compressed with flat retrolateral surfaces, with bristle combs consisting of retrosuperior series of longer macrosetae, plus retroinferior and proinferior series of shorter macrosetae; telotarsi with two rows of short macrosetae on ventral aspect, 9–13 macrosetae on telotarsus III; tibial spurs moderate to reduce on legs IV and almost reduced on legs III.

Metasoma and telson (Figs. 6–13). Metasoma and telson sparsely hirsute, macrosetae moderately short in male and longer in female, straight and reddish; metasomal segments I–III with 10 carinae, IV with 8 carinae, V with 2 carinae; segments I–III with moderate, granulate dorsolateral carinae, other carinae relatively well developed; segment IV with weakly indicated dorsolateral carinae; segment V with strong, granulate to dentate-lobate ventrolateral carinae; segments I–IV with dense granulation on all intercarinal surfaces except dorsal surfaces which are sparsely granulated in male and almost smooth in female; segment V densely granular on lateral and ventral surfaces, more coarsely so on ventral surface, granules not arranged along any traces of carinae; telson tuberculate, ventral surface sparsely, weakly granular; vesicle slightly elongated; aculeus stout, shorter than vesicle, tip of aculeus almost vertically directed.

AFFINITIES. The described features distinguish *Neobuthus fryntai* sp. nov. from all other species of the genus. According to the characters used in the key published in Kovařík et al. (2018), the new species which is also confirmed by DNA phylogeny (paper in preparation) is most similar to *N. factorio* Kovařík, Lowe, Awale, Elmi et Hurre, 2018. *Neobuthus fryntai* sp. nov. has pedipalp segments relatively slender than *N. factorio*, males with femur L/W 2.74–2.86 (2.42–2.70 in *N. factorio*), patella L/W 2.50–2.62 (2.18–2.28 in *N. factorio*), chela L/W 4.68–4.98 (4.25–4.48 in *N. factorio*).

DISCUSSION

The *Neobuthus* genus from the family Buthidae is distributed in the Horn of Africa including Eritrea, Djibouti, Ethiopia, Kenya, Somalia, and Somaliland (Figs. 44–45). The Horn of Africa is a hotspot region of the fauna had a long evolutionary history of ecological, climatic, geological, and erosional processes that could influence the evolution of the scorpion fauna (Redfield et al. 2003, Keir et al. 2013, Aghová et al. 2019). Moreover, the geographical separations of the region led by physical barriers like mountain ranges, rivers, and vegetative zones could result in the effective isolation of the *Neobuthus* genus in the region. Therefore, some species of the genus *Neobuthus* are widely spread in Somaliland see distribution (Figs. 44–45) whereas most of species are endemic there (e. g., *N. haeckeli* Kovařík, 2019, *N. solegladi* Kovařík, 2019, *N. berberensis* Hirst, 1911, *N. erigavoensis* Kovařík, Lowe, Awale, Elmi et Hurre, 2018, *N. factorio*, *N. gubanensis* Kovařík, Lowe, Awale, Elmi et Hurre, 2018, *N. maidensis* Kovařík, Lowe, Awale, Elmi et Hurre, 2018, *N. montanus* Kovařík, Lowe, Awale, Elmi et Hurre, 2018). The newly discovered *Neobuthus fryntai* sp. nov. represents one more endemic species and makes the number of known species of the genus *Neobuthus* sixteen. Somaliland corresponding former British Somaliland (British colony) host many diverse ecological zones and climatic conditions (Kovařík 2019, Salah et al. 2019) and its landscape is divided into many topo-graphical characteristics (Resource et al. 2018), including the coastal regions, the rocky mountain area in central regions (Kovařík 2019), hot sand deserts at a lower elevation, rocky semi-desert at a higher elevation, and sandy semi-desert habitats with volcanic rock (Kovařík & Lowe 2016).

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