

# A contribution on the morphometrics of the thick-clawed crayfish *Pontastacus pachypus* (Rathke, 1837) (Decapoda, Astacoidea, Astacidae)

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## Abstract

The thick-clawed crayfish *Pontastacus pachypus* (Rathke, 1837) is an endangered, and the least known, crayfish species in Europe. Currently, detailed information regarding the morphology, ecology and reproduction of thick-clawed crayfish is outdated. This study presents, for the first time, detailed photographs of the thick-clawed crayfish, and updated information on distinctive morphological characters and morphometric analysis. New specifications of the carapace and appendage morphological characteristics were established as: 1) the rostrum is long, sharply pointed and has three pairs of distinctive sub-apical lateral spines, 2) two well-developed pairs of post-orbital ridge on the carapace are ended by prominent spine; 3) each finger of chelae ends with a black sharp tip. Among the 18 morphometric indices, carapace width to the total length (CPW/TL), abdomen width to the total length (ABW/TL) and claw height to the claw width (CLH/CLW) clearly differentiate *P. pachypus* from the other representatives of *Astacus* genus (*A. colchicus* and *A. astacus*) and *P. leptodactylus* ( $P < 0.05$ ). Comparison of individual indices between *P. pachypus* and *P. leptodactylus* revealed that almost all indices differed significantly except head length to the total length (HEL/TL) and rostrum length to the total length (ROL/TL). This study contributes to the identification of the thick-clawed crayfish for the purpose of conservation and protection of its localities.

## Key Words

*Astacus astacus*, *Astacus colchicus*, morphometric indices, *Pontastacus leptodactylus*, species identification

## Introduction

The thick-clawed crayfish *Pontastacus pachypus* (Rathke, 1837) is one of the least widespread and studied native crayfish in Europe (Policar et al. 2018) primarily due to difficulties in obtaining samples and a substantial decline in population numbers (Anosov and Timofeev 2016; Bláha et al. 2017; Policar et al. 2018). Lack of knowledge about this species most probably stems from its area of distribution. Eastern European countries in general, and Russia, Kazakhstan and Ukraine, in particular, are either neglected by scientists or geopolitical difficulties, and a high degree of bureaucracy, prevent studies

from being carried out. Such a situation has resulted in a longstanding scarcity of specimens and biological material of this crayfish species. Therefore, scientists or naturalists rely on old, very often original species descriptions (Brodsky 1981), which might lack sufficient detail when compared to current standards.

Although, historically, this species was assigned to the genus *Astacus* (Fabricius, 1775) or *Caspiastacus* (Starobogatov, 1995), morphological characteristics such as abdominal somites II–IV with pleura bearing acute spines, and male pleopod II with ventral process, indicated its assignment to genus *Pontastacus* Bott, 1950 (Füreder and Machino 2002; Rogers and Thorp 2019) mentioned this species in

the updated classification of freshwater crayfish (Crandall and De Grave 2017). Moreover, most information relating to its distribution and biology is outdated and repeated from publication to publication (Füreder and Machino 2002; Pöckl et al. 2006), except for three recently published studies about thick-clawed crayfish in Ukraine (Mezhzherin et al. 2015; Bláha et al. 2017; Policar et al. 2018).

Generally, the thick-clawed crayfish is characterized as being relatively smaller (Kouba et al. 2015) than other species of the family Astacidae, such as the noble crayfish *Astacus* (Linné, 1758) and narrow-clawed crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823). In addition, it is probably the most colorful species among others in the family Astacidae, which is also one of its typical characteristics. The most accurate description is provided by Kouba et al. (2015): the carapace is relatively soft, with two pairs of well-developed postorbital ridges, the first ended by an acute spine. The rostrum has a similar shape to narrow-clawed crayfish with parallel edges and a smaller spine. Claws have a spherical shape, rounded at the end and yellow from the bottom side. During the mating season, protrusions on claws and legs might be orange or even red. The biology of this species is poorly studied. Therefore, our knowledge about ecology, reproduction, or habitat preferences is either unknown or very limited (Holdich 2002; Kouba et al. 2015). Original distribution as described in classical works of Brodsky (1981) or Starobogatov (1995) includes North Caspian and Middle-South Caspian shallow water provinces as well as deltas of big rivers in the Azov and the Black Sea drainage (Volga, Dniester and Dnieper River) in Ukraine. Nevertheless, according to the most recent studies (Kouba et al. 2015; Policar et al. 2018) the current geographic range extends to fresh- and brackish waters in eastern Europe (Ukraine, Russia) and countries surrounding the Caspian Sea (Azerbaijan, Kazakhstan and Turkmenistan) (Fig. 1).

Moreover, in natural localities, thick-clawed crayfish are usually found in sympatry with narrow-clawed crayfish. Due to fishermen's inability to distinguish endangered thick-clawed crayfish from most common narrow-clawed crayfish, both species are intensively used

for consumption. This dangerous practice may negatively affect the future occurrence and protection of this crayfish species, e.g. in Ukraine (Policar et al. 2018).

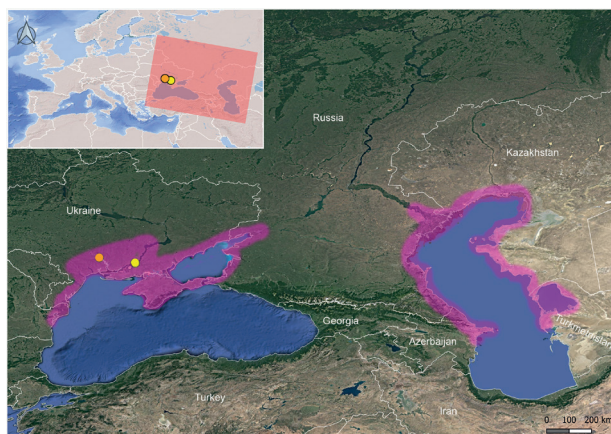
The aim of this study was (a) to describe the basic morphometric characteristics of thick-clawed crayfish and (b) to provide the distinguishing morphological characteristics enabling easy species determination from narrow-clawed crayfish in the field (c) to contrast the morphological differences of *P. pachypus* in comparison to *P. leptodactylus*, *A. colchicus* and *A. astacus*.

## Materials and methods

### Data acquisition

For this study, adult females (n=47) and males (n=51) of thick-clawed crayfish were caught in the locality of the main Dnieper River (46°46.452'N, 33°22.090'E) near Nova Kakhovka town in southern Ukraine (Policar et al. 2018) by two divers searching the river bottom for two hours. After measurements, most individuals were released back to the locality and allowed to regenerate. Additionally, freshly dead adult thick-clawed crayfish (10 females and 20 males) sold as marketable narrow-clawed crayfish were bought in the local market at the same town as before (46°47.683'N, 33°20.560'E) for analysis of weight portion of both claws and to photograph the key morphological characteristics of this species. Individuals with a total length (TL) of less than 60 mm were excluded from this study in order to avoid intraspecific comparisons between adults and juveniles, and to only analyze and observe adult crayfish's morphological parameters and characteristics (Policar et al. 2018). The dead thick-clawed crayfish were preserved in pure 96% ethanol for future scientific analysis and student education.

Individuals of *A. colchicus* (n= 51) were caught in different locations in Georgia (Blaha et al. 2021), *P. leptodactylus* (n=100) and *A. astacus* (n=81) were caught in Podolský brook, Vápenný Podol village, Czech Republic for species morphological analysis and comparison.



**Figure 1.** *Pontastacus pachypus* distribution map overlay populations sampled for this study (yellow circles) and population from the type locality (orange circle).

### Illustration and description of distinctive morphological characters for better species identification

Four freshly dead thick-clawed crayfish (two adult males and females) were photographed with a digital camera CAMEDIA C-5050 ZOOM (Olympus, Czech group Ltd.). All key distinctive morphological characters were illustrated and described in detail to facilitate better identification of this species for future field and conservation purposes.

### Morphometric measurements

All morphometric characteristics were recorded by a digital caliper to the nearest 0.1 mm. In addition, individuals of

*P. leptodactylus*, *A. astacus* and *A. colchicus* were also measured and analyzed to contrast morphological differences between species. In total, 21 morphological characteristics adopted from Sint et al. (2005), were measured for each crayfish. Bilateral parameters related to claws' asymmetry were measured on the right side, except when injuries or regenerations were observed, which significantly affected measurements. In this case, measurements were taken on the left side (Sint et al. 2005). All measurements were converted into 18 indices: CPL/CLL—length of the claw palm to the claw length; CLW/CLL—claw width to the claw length; HEL/TL—head length to the total length; CEW/TL—width of the carapace at the hind edges to the total length; CPW/TL—carapace width to the total length; ABW/TL—abdomen width to the total length; ABH/TL—abdomen height to the total length; TEW/TL—telson width to the to the total length; ROL/TL—rostrum length to the total length; CLH/CLW—claw height to the claw width; CFL/CPL—length of the claw finger to the length of the claw palm; TEL/TEW—telson length to the telson width; ROL/ROW—rostrum length to the rostrum width; ABL/TL—abdomen length to the total length; HEL/HEW—head length to the head width; CPX/CPW—carapace length (rostrum length, head length, areolar length are included) to the carapace width; CPX/TL—carapace length (rostrum length, head length, areolar length are included) to the total length; HEL/HEW—head length to the head width. Additionally, body weight in all studied thick-clawed crayfish was recorded with a professional electronic scale (Kern and Sohn GmbH, Balingen, Germany) to the nearest 0.01g. The same procedure was repeated with the weight of each claw (LCLW on the left side and RCLW on the right side) in dead crayfish bought at the market. The weight portion of both claws to the body weight (WPBC in %) was calculated according to the formula:  $(LCLW + RCLW) * 100 / BW$ . Frequency of injured, damaged and regenerated right, left or both claws were observed according to Kouba et al. (2011).

Obtained biometric data were used to calculate postorbital length (POL) as follows:  $POL = HEL + ARL$ . This parameter was further used for the first characteristic description of the carapace shape as a ratio of postorbital length (POL) and carapace width (CPW), according to Sint et al. (2005). The lateral curvature of the carapace was used as a second parameter calculated according to Sint et al. (2005)  $\alpha = \arctan(HEL / (CPW - HEW) / 2) + \arctan(ARL / (CPW - CEW) / 2)$  as the angle at carapace width (CPW) between head width (HEW) and carapace width at the hind edges (CWHE).

## Statistical analysis

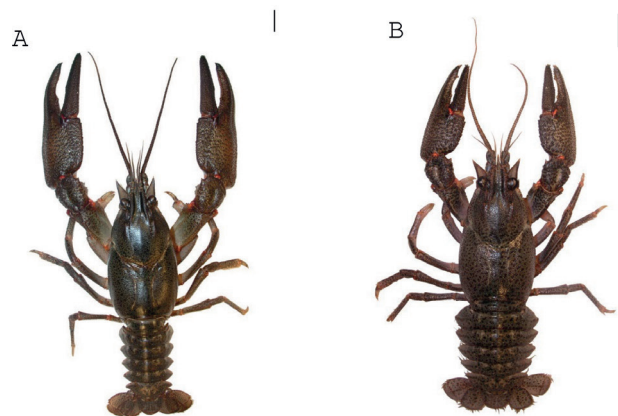
To compare differences in biometric data between sexes of *P. pachypus*, a separate model for each biometric parameter was run. Data were firstly checked for normality and homoscedasticity using the Shapiro-Wilk test and the Bartlett test, respectively. Subsequently, either ANOVA or non-parametric Kruskal-Wallis tests were performed. Statistical significance was determined at  $P < 0.05$ . Multivari-

ate analysis was employed to test the morphological differences of *P. pachypus* in comparison with the most closely related crayfish species *P. leptodactylus*, *A. colchicus* and *A. astacus*. Indices were used as continuous variables, whereas crayfish species were used as a discrete factor. Firstly, a detrended correspondence analysis was run to find a length of gradient and thus choose either linear or unimodal analysis. A linear analysis (Principal Component Analysis-PCA) was performed based on the length of the gradient. All statistical analysis was performed using the R program (R Core Team, Vienna, Austria).

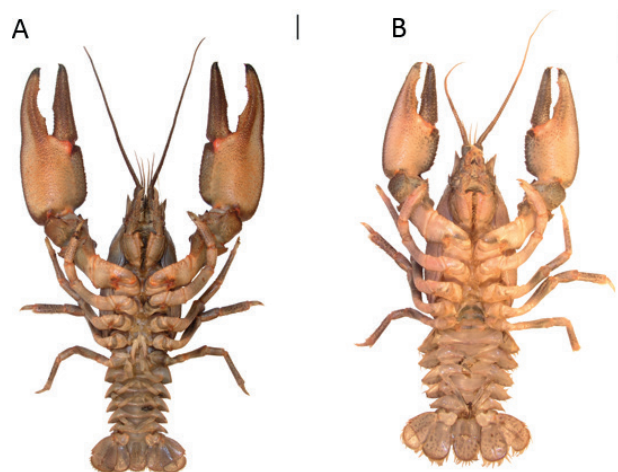
## Results

### Morphological description of *P. pachypus*

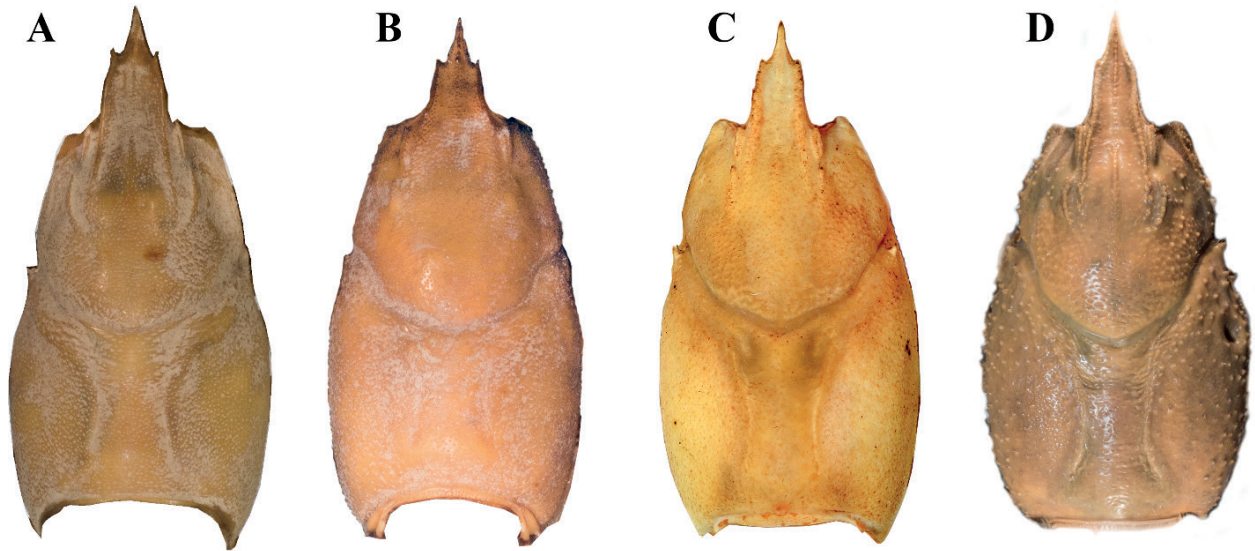
The color in live observations of the dorsal side of the body is variable (dark brown, grayish or greenish-brown), however the ventral side of the body is light in color (beige, yellow or whitish) (Figs 2, 3). The carapace is relatively smooth. Two pairs of postorbital ridges are well-developed and each ended by a prominent spine (Fig. 4C). The rostrum is long with more or less parallel edges covered with small spines. The edges of the rostrum have



**Figure 2.** Dorsal view of total body in thick-clawed crayfish (*P. pachypus*) male (A), female (B). Scale bars: 10 mm.

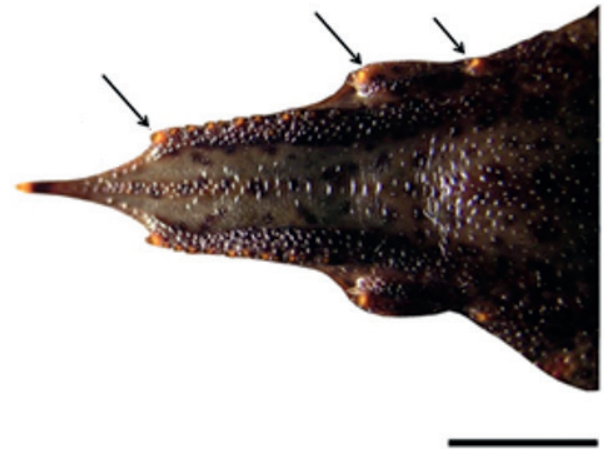


**Figure 3.** Ventral view of total body in thick-clawed crayfish (*P. pachypus*) male (A), female (B). Scale bars: 10 mm.



**Figure 4.** Dorsal view on carapace of *A. colchicus* (A), *A. astacus* (B), *P. pachypus* (C), *P. leptodactylus* (D).

three pairs of distinctive sub-apical lateral spines (Fig. 5), which are noticeable and sharply pointed. The chelae have the same color as the crayfish body. The dorsal surface of the chelae is relatively rough (Fig. 6), while the ventral surface is smooth. The chelae are wider and more robust than in other indigenous European crayfish (Table 1). A visible furrow in the fixed finger, which is bordered by two spines on each side, is typical for this crayfish species. Both fingers of the chelae end in black sharp tips (Fig. 6). The pleura of the abdominal somites is wedge-shaped without spinules at the ventral ends (Fig. 7D, E).



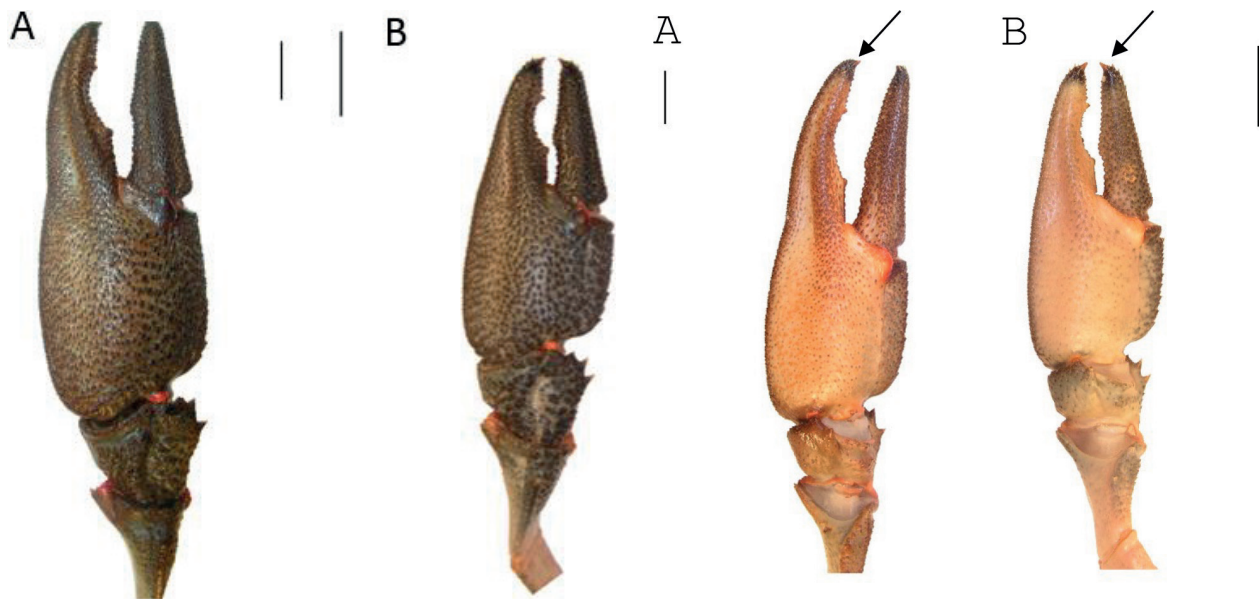
**Figure 5.** Detailed rostrum shape with three pairs of main sub-apical lateral spines (arrows) of thick-clawed crayfish (*P. pachypus*). Scale bars: 10 mm.

## Morphometric analysis

Table 1 summarized the comparison of the morphometric indices of *P. pachypus*, *P. leptodactylus*, *A. astacus* and

**Table 1.** Morphometric indices of *P. pachypus*, *P. leptodactylus*, *A. astacus* and *A. colchicus* with mean values and standard deviation (SD). Values with different letters are significantly different (lower case for males; capital letters for females) ( $P < 0.05$ ).

| Morphometric indices | <i>P. pachypus</i>     |                         | <i>P. leptodactylus</i> |                        | <i>A. astacus</i>      |                        | <i>A. colchicus</i>     |                         |
|----------------------|------------------------|-------------------------|-------------------------|------------------------|------------------------|------------------------|-------------------------|-------------------------|
|                      | Male (mean±SD)         | Female (mean±SD)        | Male (mean±SD)          | Female (mean±SD)       | Male (mean±SD)         | Female (mean±SD)       | Male (mean±SD)          | Female (mean±SD)        |
| CPL/CLL              | 0.36±0.04 <sup>a</sup> | 0.36±0.02 <sup>A</sup>  | 0.29±0.03 <sup>c</sup>  | 0.32±0.06 <sup>B</sup> | 0.32±0.03 <sup>b</sup> | 0.32±0.03 <sup>B</sup> | 0.35±0.02 <sup>a</sup>  | 0.34±0.03 <sup>AB</sup> |
| CLW/CLL              | 0.37±0.03 <sup>b</sup> | 0.41±0.05 <sup>A</sup>  | 0.34±0.03 <sup>c</sup>  | 0.42±0.03 <sup>A</sup> | 0.41±0.03 <sup>a</sup> | 0.42±0.03 <sup>A</sup> | 0.40±0.04 <sup>a</sup>  | 0.39±0.03 <sup>A</sup>  |
| HEL/TL               | 0.22±0.01 <sup>b</sup> | 0.20±0.01 <sup>AC</sup> | 0.22±0.02 <sup>b</sup>  | 0.20±0.02 <sup>A</sup> | 0.23±0.01 <sup>a</sup> | 0.22±0.02 <sup>B</sup> | 0.20±0.01 <sup>c</sup>  | 0.19±0.00 <sup>c</sup>  |
| CEW/TL               | 0.21±0.01 <sup>a</sup> | 0.20±0.01 <sup>A</sup>  | 0.20±0.01 <sup>a</sup>  | 0.19±0.01 <sup>B</sup> | 0.20±0.01 <sup>a</sup> | 0.20±0.01 <sup>A</sup> | 0.19±0.01 <sup>b</sup>  | 0.18±0.01 <sup>c</sup>  |
| CPW/TL               | 0.28±0.02 <sup>a</sup> | 0.26±0.01 <sup>A</sup>  | 0.26±0.02 <sup>b</sup>  | 0.24±0.01 <sup>B</sup> | 0.26±0.01 <sup>b</sup> | 0.25±0.01 <sup>B</sup> | 0.25±0.02 <sup>c</sup>  | 0.23±0.01 <sup>c</sup>  |
| ABW/TL               | 0.23±0.01 <sup>a</sup> | 0.29±0.01 <sup>A</sup>  | 0.21±0.03 <sup>b</sup>  | 0.27±0.02 <sup>B</sup> | 0.21±0.01 <sup>b</sup> | 0.24±0.01 <sup>c</sup> | 0.22±0.01 <sup>b</sup>  | 0.24±0.02 <sup>c</sup>  |
| ABH/TL               | 0.08±0.01 <sup>d</sup> | 0.08±0.01 <sup>C</sup>  | 0.09±0.01 <sup>c</sup>  | 0.10±0.02 <sup>B</sup> | 0.10±0.01 <sup>b</sup> | 0.11±0.01 <sup>B</sup> | 0.15±0.01 <sup>a</sup>  | 0.15±0.01 <sup>A</sup>  |
| TEW/TL               | 0.12±0.00 <sup>a</sup> | 0.12±0.00 <sup>B</sup>  | 0.12±0.01 <sup>a</sup>  | 0.13±0.01 <sup>A</sup> | 0.12±0.01 <sup>a</sup> | 0.12±0.01 <sup>B</sup> | 0.12±0.00 <sup>a</sup>  | 0.12±0.00 <sup>B</sup>  |
| ROL/TL               | 0.13±0.01 <sup>b</sup> | 0.13±0.01 <sup>B</sup>  | 0.13±0.01 <sup>b</sup>  | 0.13±0.01 <sup>B</sup> | 0.14±0.01 <sup>a</sup> | 0.14±0.01 <sup>A</sup> | 0.13±0.01 <sup>b</sup>  | 0.14±0.01 <sup>A</sup>  |
| CLH/CLW              | 0.58±0.04 <sup>a</sup> | 0.59±0.03 <sup>A</sup>  | 0.57±0.04 <sup>b</sup>  | 0.52±0.04 <sup>C</sup> | 0.55±0.04 <sup>c</sup> | 0.52±0.03 <sup>C</sup> | 0.57±0.10 <sup>b</sup>  | 0.54±0.03 <sup>B</sup>  |
| CFL/CPL              | 1.49±0.18 <sup>c</sup> | 1.45±0.13 <sup>C</sup>  | 2.03±0.22 <sup>a</sup>  | 1.81±0.18 <sup>A</sup> | 1.72±0.19 <sup>b</sup> | 1.78±0.23 <sup>A</sup> | 1.61±0.14 <sup>b</sup>  | 1.64±0.13 <sup>B</sup>  |
| TEL/TEW              | 1.18±0.07 <sup>a</sup> | 1.24±0.06 <sup>A</sup>  | 1.14±0.08 <sup>b</sup>  | 1.17±0.10 <sup>B</sup> | 1.20±0.07 <sup>a</sup> | 1.20±0.07 <sup>A</sup> | 1.20±0.09 <sup>a</sup>  | 1.18±0.07 <sup>B</sup>  |
| ROL/ROW              | 2.10±0.29 <sup>b</sup> | 2.10±0.24 <sup>A</sup>  | 1.88±0.25 <sup>c</sup>  | 2.04±0.28 <sup>A</sup> | 1.95±0.18 <sup>c</sup> | 1.95±0.20 <sup>A</sup> | 2.28±0.41 <sup>a</sup>  | 2.12±0.37 <sup>A</sup>  |
| ABL/TL               | 0.35±0.02 <sup>b</sup> | 0.38±0.02 <sup>A</sup>  | 0.36±0.05 <sup>a</sup>  | 0.38±0.02 <sup>A</sup> | 0.35±0.02 <sup>b</sup> | 0.38±0.02 <sup>A</sup> | 0.36±0.01 <sup>a</sup>  | 0.37±0.01 <sup>B</sup>  |
| CPX/TL               | 0.55±0.03 <sup>a</sup> | 0.51±0.02 <sup>B</sup>  | 0.53±0.02 <sup>b</sup>  | 0.50±0.02 <sup>B</sup> | 0.53±0.02 <sup>b</sup> | 0.52±0.02 <sup>A</sup> | 0.50±0.02 <sup>c</sup>  | 0.49±0.02 <sup>B</sup>  |
| CPX/CPW              | 1.97±0.18 <sup>b</sup> | 1.92±0.11 <sup>B</sup>  | 2.03±0.20 <sup>ab</sup> | 2.04±0.13 <sup>A</sup> | 2.06±0.11 <sup>a</sup> | 2.08±0.10 <sup>A</sup> | 2.00±0.09 <sup>ab</sup> | 2.05±0.09 <sup>A</sup>  |
| HEL/HEW              | 1.97±0.18 <sup>a</sup> | 1.38±0.10 <sup>B</sup>  | 1.47±0.12 <sup>b</sup>  | 1.44±0.16 <sup>A</sup> | 1.49±0.10 <sup>b</sup> | 1.48±0.09 <sup>A</sup> | 1.38±0.06 <sup>c</sup>  | 1.36±0.06 <sup>B</sup>  |



**Figure 6.** Dorsal view of thick-clawed crayfish (*P. pachypus*) chelae male (A), female (B). Scale bars: 10 mm.

**Figure 7.** Ventral view of thick-clawed crayfish (*P. pachypus*) chelae male (A), female (B) with black sharp tip (arrow) Scale bars: 10 mm.

**Table 2.** Morphological characteristics concerning claws in males and females of thick-clawed crayfish (*P. pachypus*). Data are expressed as means  $\pm$  standard deviations. Different letters in same row indicate significant differences in normalized data between females and males ( $P < 0.05$ ).

| Parameter  | Males                                    |      |      | Females  |      |      |
|--|--|------|------|--|------|------|
|  | (n= 51; except LCLW, RCLW and WPBC n=20) | Min. | Max. | (n=47; except CLL, CLW, CLH, CPL and CFL n= 46 and LCLW, RCLW and WPBC n=10) | Min. | Max. |
| Claw length – CLL (mm)                                       | 51.9 $\pm$ 12.77 <sup>a</sup>            | 30.0 | 77.6 | 32.7 $\pm$ 4.98 <sup>b</sup>   | 24.4 | 45.3 |
| Claw width – CLW (mm)  | 19.2 $\pm$ 4.45 <sup>a</sup>             | 11.8 | 28.4 | 13.3 $\pm$ 2.53 <sup>b</sup>   | 5.6  | 17.1 |
| Claw height – CLH (mm)                                       | 11.4 $\pm$ 2.85 <sup>a</sup>             | 6.6  | 16.3 | 8.7 $\pm$ 2.40 <sup>b</sup>  | 4.8  | 19.5 |
| Length of the claw palm – CPL (mm)                           | 18.7 $\pm$ 5.20 <sup>a</sup>             | 10.8 | 32.1 | 11.7 $\pm$ 1.99 <sup>b</sup>   | 8.1  | 16.6 |
| Length of the claw finger – CFL (mm)                         | 26.6 $\pm$ 6.44 <sup>a</sup>             | 15.5 | 40.0 | 16.9 $\pm$ 2.47 <sup>b</sup>   | 12.4 | 24.2 |
| Left claw weight – LCLW (g)                                  | 9.0 $\pm$ 2.83 <sup>a</sup>              | 4.1  | 13.8 | 3.0 $\pm$ 0.60 <sup>b</sup>  | 1.9  | 3.6  |
| Right claw weight – RCLW (g)                                 | 9.3 $\pm$ 3.24 <sup>a</sup>              | 4.2  | 15.7 | 3.0 $\pm$ 0.60 <sup>b</sup>  | 2.0  | 4.0  |
| Weight portion of both claws – WPBC (%)                      | 38.2 $\pm$ 2.40 <sup>a</sup>             | 31.4 | 40.9 | 26.4 $\pm$ 1.85 <sup>b</sup>   | 24.0 | 29.5 |
| Lengthwise portion of claw length CLL to total length TL (%) | 55.6 $\pm$ 6.86 <sup>a</sup>             | 43.0 | 73.1 | 39.4 $\pm$ 3.20 <sup>b</sup>   | 29.9 | 45.9 |
| Frequency of missing right claw (%)                          | 1.9                                      | –    | –    | 6.4  | –    | –    |
| Frequency of missing left claw (%)                           | 13.7                                     | –    | –    | 23.4   | –    | –    |
| Frequency of missing both claws (%)                          | 0  | –    | –    | 2.1  | –    | –    |

*A. colchicus*. The morphometric analysis revealed CPW/TL, ABW/TL and CLH/CLW were significantly higher in *P. pachypus* compared with others (Table 1). Comparison of individual indices among different male crayfish species revealed almost all indices differed significantly except TEW/TL. Among the 18 studied morphometric indices, five were statistically highest in *P. pachypus* compared with others. While a comparison of individual indices among different female crayfish species revealed almost all indices differed significantly except CLW/CLL and TEW/TL. Comparison of individual indices between males of *P. pachypus* and *P. leptodactylus* revealed almost all indices differed significantly except HEL/TL, CEW/TL, TEW/TL, ROL/TL and CPX/CPW. A similar trend was observed between females of *P. pachypus* and *P. leptodactylus*, where almost all indices differed significantly except CLW/CLL, HEL/TL, ROL/TL, ROL/ROW, ABL/TL and CPX/TL (Table 1). Furthermore, we employed PCA to determine which

morphometric traits were most efficient at discriminating the four species (Fig. 8). The PCA revealed that two principal components account for 34% of the total variation. PC1 and PC2 were largely influenced by the CPX/TL, CPW/TL, HEL/HEW, HEL/TL, CFL/CPL and CPL/CLL (Fig. 9).

Males of thick-clawed crayfish possess larger claws than females; as predicted, in our study males of *P. pachypus* had significantly larger claws (Table 2); rostrum, head, abdomen, telson, carapace (Table 3); total length and body weight (Table 4) than females.

## Discussion

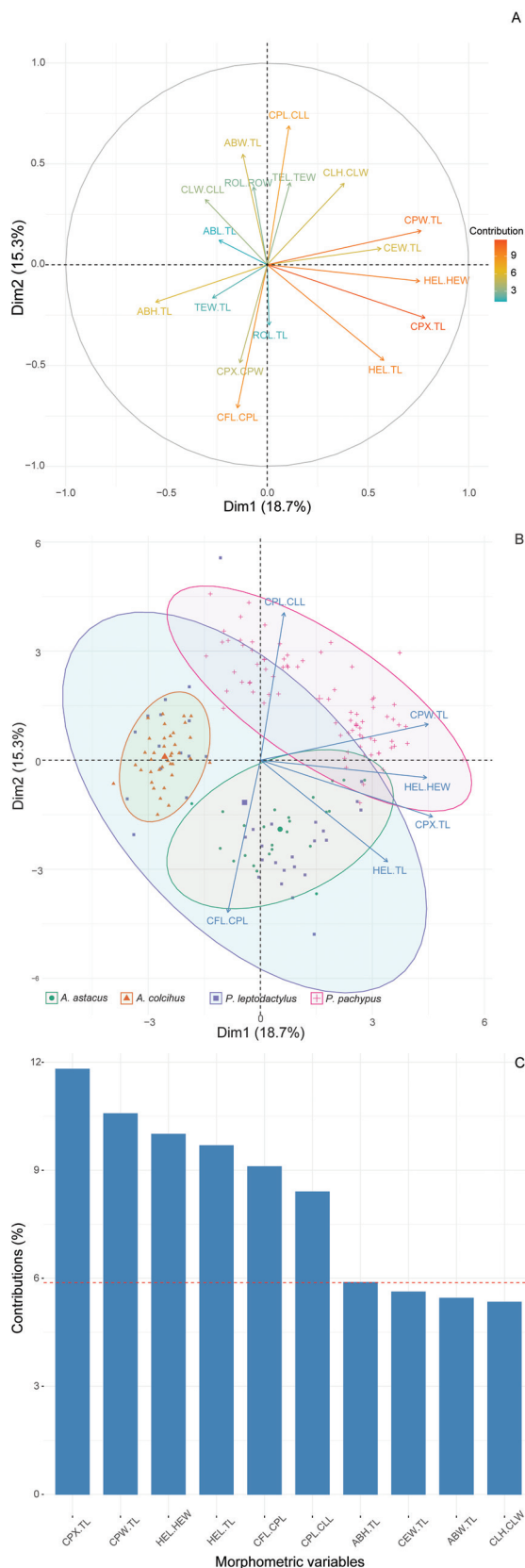
This study was conducted to update the morphological description of the thick-clawed crayfish based on specimens collected in the Dnieper River, Ukraine. The characteristics used to distinguish the thick-clawed crayfish with



**Figure 8.** Pleura of abdominal somites of *A. colchicus* male (A), female (B); *A. astacus* male (C); *P. pachypus* male (D), female (E); *P. leptodactylus* male (F).

**Table 3.** Morphological characteristics concerning rostrum, head, abdomen, telson and carapace in males and females of thick-clawed crayfish (*P. pachypus*). Data are expressed as means  $\pm$  standard deviations. Different letters in same row indicate significant differences in normalized data between females and males ( $P < 0.05$ ).

| Parameter   | Males (n= 51)                |      |      | Females (n=47)                |      |      |
|---|------------------------------|------|------|-------------------------------|------|------|
|   | Mean $\pm$ S.D.              | Min. | Max. | Mean $\pm$ S.D.               | Min. | Max. |
| Rostrum length – ROL (mm)                         | 11.8 $\pm$ 1.52 <sup>a</sup> | 8.8  | 15.1 | 10.3 $\pm$ 1.17 <sup>b</sup>  | 7.2  | 12.9 |
| Rostrum width – ROW (mm)                          | 5.7 $\pm$ 0.92 <sup>a</sup>  | 3.2  | 7.8  | 5.0 $\pm$ 0.74 <sup>b</sup>   | 3.9  | 7.5  |
| Head length – HEL (mm)                            | 20.0 $\pm$ 3.27 <sup>a</sup> | 12.9 | 26.6 | 16.4 $\pm$ 1.95 <sup>b</sup>  | 12.2 | 21.6 |
| Head width – HEW (mm)                             | 13.9 $\pm$ 1.95 <sup>a</sup> | 10.1 | 17.8 | 11.8 $\pm$ 1.35 <sup>b</sup>  | 9.5  | 16.4 |
| Areolar length – ARL (mm)                         | 19.0 $\pm$ 3.13 <sup>a</sup> | 12.1 | 26.1 | 15.1 $\pm$ 1.67 <sup>b</sup>  | 11.5 | 19.6 |
| Areolar width – ARW (mm)                          | 6.8 $\pm$ 1.1 <sup>a</sup>   | 4.9  | 9.1  | 6.0 $\pm$ 0.66 <sup>b</sup>   | 4.7  | 7.7  |
| Abdomen length – ABL (mm)                         | 32.5 $\pm$ 5.0 <sup>a</sup>  | 22.8 | 42.7 | 30.1 $\pm$ 3.20 <sup>b</sup>  | 25.6 | 39.4 |
| Abdomen width – ABW (mm)                          | 21.6 $\pm$ 3.2 <sup>a</sup>  | 15.5 | 27.8 | 24.1 $\pm$ 3.36 <sup>b</sup>  | 16.9 | 34.0 |
| Abdomen height – ABH (mm)                         | 7.8 $\pm$ 1.6 <sup>a</sup>   | 4.1  | 12.0 | 6.7 $\pm$ 1.38 <sup>b</sup>   | 4.8  | 11.9 |
| Telson length – TEL (mm)                          | 12.8 $\pm$ 1.9 <sup>a</sup>  | 9.2  | 16.1 | 12.5 $\pm$ 1.7 <sup>b</sup>   | 9.3  | 19.5 |
| Telson width – TEW (mm)                           | 10.8 $\pm$ 1.6 <sup>a</sup>  | 7.4  | 13.6 | 10.1 $\pm$ 1.13 <sup>b</sup>  | 7.8  | 13.6 |
| Carapace width – CPW (mm)                         | 26.2 $\pm$ 4.3 <sup>a</sup>  | 17.7 | 33.6 | 21.8 $\pm$ 2.42 <sup>b</sup>  | 16.9 | 30.0 |
| Carapace width at the cervical groove – CWCG (mm) | 21.5 $\pm$ 3.2 <sup>a</sup>  | 14.5 | 26.7 | 18.1 $\pm$ 2.30 <sup>b</sup>  | 14.1 | 24.2 |
| Carapace width at the hind edges – CWHE (mm)      | 18.9 $\pm$ 2.8 <sup>a</sup>  | 13.8 | 24.1 | 16.8 $\pm$ 1.98 <sup>b</sup>  | 13.6 | 23.3 |
| Carapace height – CPH (mm)                        | 21.4 $\pm$ 3.5 <sup>a</sup>  | 15.2 | 28.1 | 18.70 $\pm$ 2.17 <sup>b</sup> | 14.7 | 25.3 |



**Figure 9.** Principal Component Analysis (PCA) of morphometric data of *A. colchicus*, *A. astacus*, *P. pachypus*, *P. leptodactylus*. (A) Biplot of the variable PCA is shown based on the morphometric data of four different species (B) Biplot of the individual PCA is shown based on the morphometric data of four different species (C) Relative contribution of morphometric variables to Dim-1 and Dim-2 to discriminate four species.

**Table 4.** Morphological characteristics concerning total length, body weight and calculated postorbital length, lateral curvature of the carapace and Fulton's condition coefficient in males and females of thick-clawed crayfish (*P. pachypus*). Data are expressed as means  $\pm$  standard deviations. Different letters in same row indicate significant differences in normalized data between females and males ( $P < 0.05$ ).

| Parameter   | Males (n= 51)                 |       |       | Females (n=47)                |       |       |
|---|-------------------------------|-------|-------|-------------------------------|-------|-------|
|   | Mean $\pm$ S.D.               | Min.  | Max.  | Mean $\pm$ S.D.               | Min.  | Max.  |
| Total length – TL (mm)  | 92.2 $\pm$ 13.30 <sup>a</sup> | 66.4  | 114.2 | 82.8 $\pm$ 8.40 <sup>b</sup>  | 66.0  | 107.5 |
| Body weight – BW (g)  | 35.5 $\pm$ 16.60 <sup>a</sup> | 10.8  | 71.7  | 18.3 $\pm$ 6.19 <sup>b</sup>  | 7.5   | 39.1  |
| Postorbital length – POL (mm)                                     | 38.9 $\pm$ 6.30 <sup>a</sup>  | 25.0  | 51.1  | 31.5 $\pm$ 3.50 <sup>b</sup>  | 23.7  | 41.1  |
| Carapace shape – Ratio POL:CPW                                    | 1.49 $\pm$ 0.06 <sup>a</sup>  | 1.28  | 1.64  | 1.44 $\pm$ 0.10 <sup>a</sup>  | 1.27  | 1.77  |
| Carapace shape – Lateral curvature of the carapace – $\alpha$ (°) | 152.3 $\pm$ 2.80 <sup>a</sup> | 144.4 | 158.2 | 153.5 $\pm$ 4.10 <sup>b</sup> | 145.9 | 168.0 |

drawings or pictures are described in a simple way in Pöckl et al. (2006). With the collection of thick-clawed crayfish studied and the analysis of its morphology, it became clear that the previous descriptions (Holdich 2002; Pöckl et al. 2006; Kouba et al. 2015) of the species were incomplete.

Even though the color of the thick-clawed crayfish is more distinct among the European native species, it shares some similarities with congeners. For instance, the color of the dorsal side of the carapace is similar to noble crayfish, and the ventral side is more similar to the narrow-clawed crayfish or white-clawed crayfish *Austropotamobius palipes* (Lereboullet 1858). Although the color of crayfish is greatly affected by the environment (Kouba et al. 2015), the described pattern was typical for all examined specimens. In the present study, the specimens of the thick-clawed crayfish collected from the Dnieper River (46°46.452'N, 33°22.090'E) near Nova Kakhovka town in southern Ukraine were dark brown and sometimes grayish or greenish-brown, which was consistent with the previous studies (Pöckl et al. 2006). The surface of carapace was found relatively smooth and similar to the noble crayfish. The earlier studies reported that only the first or both pairs of postorbital ridges are well developed (Holdich 2002; Pöckl et al. 2006). Contrary to previous records (Kouba et al. 2015), we observed that both well-developed pairs of postorbital ridges are ended by a sharp spine (Fig. 4). Moreover, we found the morphological differences in rostrum compared to the characteristics published earlier (Starobogatov 1995; Pöckl et al. 2006). Contrary to more or less parallel borders with small tubercles and spines, we found the rostrum with three prominent and distinctively visible pairs of spines (Fig. 5). Furthermore, these three pairs of spines were not observed in *P. leptodactylus*, *A. astacus* and *A. colchicus*. It would be necessary to analyze more of the population to confirm if this characteristic is typical not only for this particular population from the Dnieper River.

The description of fingers of chelae is one of the key characteristics distinguishing *P. pachypus* from *P. leptodactylus*. The former one has robust fingers of che-

lae with a marked incision between tubercles on the cutting edge of the fixed finger, strongly resembling the chela structure of *A. astacus*. In the present study, however, we discovered that *P. pachypus* has each finger of chelae ending with a narrow black strip before the prominent spine (Fig. 7). In contrast, no such pattern was reported for *P. leptodactylus*, *A. astacus* and *A. colchicus*. Another helpful characteristic to distinguish between genera *Astacus* and *Pontastacus* is the talon on the second male gonopod being present in *Pontastacus* representatives only. In addition, the ends of abdominal pleura II–IV being pointed is a further distinguishing feature for *Pontastacus* species (Fig. 8). Furthermore, the comparison of the morphometric indices of *P. pachypus*, *P. leptodactylus*, *A. astacus* and *A. colchicus* were analyzed in this study. Among the 18 morphometric indices, carapace width to the total length (CPW/TL), abdomen width to the total length (ABW/TL) and claw height to the claw width (CLH/CLW) clearly differentiated *P. pachypus* from the other representatives of *Astacus* genus and *P. leptodactylus*. Comparison of individual indices between *P. pachypus* and *P. leptodactylus* revealed almost all indices differed significantly except head length to the total length (HEL/TL) and rostrum length to the total length (ROL/TL). The PCA analysis showed several characteristics were most efficient at discriminating four crayfish species, in particular CPX/TL, CPW/TL, HEL/HEW, HEL/TL, CFL/CPL and length of the claw palm to the claw length CPL/CLL.

## Conclusion

This study provides an update of the morphological characteristics of endangered, and the least known, thick-clawed crayfish *Pontastacus pachypus* in order to assist better identification of this species. The color photographs of both sexes documented the most noticeable features linked to the rostrum, postorbital ridges and tips of the claw's fingers. Our study pointed out the lack of information about the current distribution of this species, which might disappear from most of its distributional range in Ukraine. We want to highlight the need to increase public awareness about this endangered species.

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