RESEARCH ARTICLE



Withius hispanus (L. Koch, 1873) new to the fauna of Iran: with an identification key of *Withius* Kew, 1911 in the Palaearctic (Pseudoscorpione; Withiidae)

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Abstract

Withius Kew, 1911 (Pseudoscorpionida: Withiidae) is a poorly known taxon comprising 46 described species that are cosmopolitan and found in most parts of the world. The diversity of *Withius* in Iran is not well understood. So far, only two species have been recorded from Iran: *W. leggi* from Mazandaran Province and *W. nanus* from Khorasan-e-Razavi Province, located in the north and northeast of Iran, respectively. In this study, *Withius hispanus* (L. Koch, 1873) is recorded for the first time from Iran. Six adult specimens of *W. hispanus* were collected under tree bark in Gilan Province, northern Iran. Moreover, a detailed description and illustrations of *W. hispanus* are provided. Additionally, we present a detailed description of the male genitalia of *W. hispanus* and compare it with other congeners in the Palaearctic region. The study also presents an updated identification key for the *Withius* species in the Palaearctic, developed based on various numeric and multistate characters using DELTA software.

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INTRODUCTION

The family Withiidae Chamberlin, 1931 is one of the four cheliferoid families which are distributed throughout the world, although it is most diverse in tropical and sub-tropical biotopes (Harvey, 2015). Based on World Pseudoscorpiones Catalog, 2024, this family includes 36 genera and 171 species worldwide. The genus *Withius* Kew, 1911 includes 46 extant species. The genus is widely distributed worldwide (Harvey, 2015). Members of this taxon are found in the Afrotropical realm (24 species), the Nearctic (three species), Indo-Malaya (four species), the Australasia and Oceania realms (two species) and the Palaearctic realm (eight species). The Palaearctic species are as follows: *W. despaxi* Vachon, 1937, *W. hispanus* (L. Koch, 1873), *W. faunus* (Simon, 1879), *W. japonicus* Morikawa, 1954, *W. nanus* Mahnert, 1988, *W. leggi* Nassirkhani, 2022, *W. neglectus* (Simon, 1878) and *W. piger* (Simon, 1878) (World Pseudoscorpiones Catalog, 2024). Two species of the genus *Withius* have been recorded in Iran so far: *W. nanus* was recorded by Nassirkhani and Hamidi (2015) from from north-eastern Iran, Khorasan-e-Razavi province which was attached to the hairs of Goodwin's brush-tailed mouse, *Calomyscus elburzensis* Goodwin, 1938 and *W. leggi* was described from north of Iran, Mazandaran Province by Nassirkhani (2022).

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FIGURE 1. A) The location of the Gilan province (study area) in Iran; B) the sampling locality.

Withius hispanus was originally raised by Koch (1873) as *Chelifer hispanus* from Spain. Chamberlin (1923) recognized *Withius* which regarded as a subgenus of the *Chelifer* as a genus based on the following characters: legs I and II femoral articulation practically perpendicular to long axis of femora; fingers of palps without accessory teeth; claws simple; rallum of chelicera with four blades. Chamberlin (1932) recognized two subfamilies for Cheliferidae. Withiinae and Cheliferinae. Weygoldt (1970) separated Withiidae from Cheliferidae based on sperm transfer behavior and spermatophores.

The aim of this paper is to redescribe *W. hispanus* from Iran based on morphological characteristics and genital structure and provide an identification key for the Palaearctic species.

MATERIAL AND METHODS

Kiashahr port is located in Gilan Province, northern Iran (Fig.1). In the present study the specimens were collected using tweezer under bark of the alder tree. The collected specimens were preserved in ethanol 75%, cleared by lactic acid 70% and permanently mounted on microscope slides using Swann's medium, and studied and illustrated with an Olympus CH–2 compound microscope equipped with a drawing tube. All measurements are in millimeters and measured with Nikon MM-40 measuring microscope. Measurements and morphological nomenclatures were adapted from Beier (1932), Chamberlin (1931), Harvey (1992) and Judson (2007). The male genital morphological nomenclature follows Heurtault (1971a) and Harvey (2015). The specimens are deposited in the pseudoscorpion collection at the Zoological Museum, Ferdowsi University of Mashhad, Iran (ZMFUM).

To create a conventional key, we found that using 13 characters was enough to distinguish between the different taxa (13 indata, 13 included, 11 in key). In order to prioritize accuracy, we assigned a higher reliability value (8) to the external characters when generating the conventional key. The character reliability in DELTA for characters 1-13 was as follows: 1,8; 2-3,7; 4,6; 5-6,5; 7,7; 8-13,8.

Abbreviations

eb = external basal; esb = external sub-basal; ib = internal basal; isb = internal sub-basal; ist = internal sub-terminal; est = external sub-terminal; it = internal terminal; et = external terminal; t = terminal; st = sub-terminal; b = basal; sb = sub-basal. Also, the following abbreviations are used: mm = millimeter; L = length and W = width. The following abbreviations are used for the male genitalia: ca, chitinized arch; ejca, ejaculatory canal atrium; la, lateral apodeme; spi, spicules; vd, ventral diverticulum.



FIGURE 2. Male W. hispanus: A) Dorsal view; B) Ventral view.



FIGURE 3. Male *W. hispanus*. A) Carapace with transverse furrows, dorsal view, arrows show the details of granulation and setae lyrifissure; B) Left chelicera with galea, dorsal view; C) chelicera with serrula exterior, dorsal view; D) Rallum with four blades.

RESULTS FAMILY WITHIIDAE CHAMBERLIN, 1931 SUBFAMILY WITHIINAE CHAMBERLIN, 1931 GENUS WITHIUS KEW, 1911

The genus *Withius* is characterized by the combination of following characters: Carapace densely granulated, longer than wide, with two distinct transverse furrows, bearing a pair of eyes; tergite divided, granulated; presence of patches of glandular setae on sternites IV-IX or X in males; rallum with four blades; palps moderately slender, granulated (summarized in Beier, 1963). In some species of the family Withiidae e.g. *W. hispanus*, *W. faunus*, *W. japonicus* and *W. neglectus*, the male genitalia are formed as elongated and triangular lateral apodemes. Other species have shortened lateral apodemes that are more similar to the genitalia structure which are common in other cheliferoids (Harvey, 2015).

Withius hispanus (L. Koch, 1873)

Synonym(s): W. lohmanderi Kobakhidze, 1965.

Material examined: 63° (ZMFUM-PSC-3001-3006), Iran: Gilan Province, Astaneh-ye Ashrafiyeh County, Kiashahr port, wooden bridge ($37^{\circ}25'30''N$, $49^{\circ}56'56.31''E$), 24.iii.2023, under tree bark, leg. Z. Latifi.

Diagnosis: *Withius hispanus* differs from other congeners by the following combination of characters: carapace granulated; with two distinct transverse furrows; hand with five setae; galea with five, minute rami; rallum with four blades, the furthest blade with several lateral denticulations; palpal femur and patella granulated; the surface of the chelal hand granulated; fixed and movable fingers of chela entirely smooth; fixed chelal finger with 21-23 teeth and movable chelal finger with 19-20 teeth; lateral apodemes long and triangular, very close together in the posterior region; dorsal diverticulum and median diverticulum approximately equal; ejaculatory canal extends to the abdomen; size of the pedipalp is as follows: femur L/W 2.66-2.72, patella L/W 2.16-2.21, chela L. (with pedicel) 0.93-0.95, hand (without pedicel) L/W 1.60, movable chelal finger L. 0.38-0.41mm.Description

Carapace: reddish brown, lateral margins darker; granulate; anterior margin straight; longer than wide, L/W 1.02-1.04; with one pair of eyes, two distinct transverse furrows present, both of them partly straight, the anterior transverse furrows distinct, the subbasal indistinct, situated close to posterior margin of carapace; with 51 setae, anterior margin with 6 setae, 17 setae in front of anterior furrow, 21 between furrows and posterior margin with 7 setae (Fig. 3A).

Tergites: lighter in color than carapace; granulated; I-X divided, XI undivided; setae with three or four apical denticulations; chaetotaxy of tergites I–X (left-right hemisternite): 3+3: 4+4: 4+4: 3+3: 7+7: 7+7: 8+7: 8+7: 8+6: 8+6; tergite XI with two long tactile setae and 10 normal setae; lyrifissures on half tergites I-X (left-right hemisternite): 3+3: 3+3: 2+3: 3+3: 2+3: 3+3: 2+3: 3+3: 2+2.

Sternites: lighter in color than tergites; entirely smooth; lightly sclerotized; II partly divided, III–X divided, XI not divided; sternite II with 11 setae; chaetotaxy sternites III–IX (right+left hemisternite): 4+4:5+6:7+7: 8+8: 8+8: 8+8: sternite X with 6+6 normal setae and two long tactile setae; sternite XI with six setae and four tactile setae.

Chelicera: brown; small; slightly sclerotized; hand with five setae; galea with five, minute rami; galeal setae present (Fig. 3B); serrula exterior with 19 blades (Fig. 3C); lamina exterior presents; rallum with four blades, the furthest blade with several lateral denticulations (Fig. 3D); fixed finger with five teeth; movable finger with one curved apical lobe and one small sub-apical tooth.

Pedipalps: reddish brown; granulated; pedipalp distinctly lighter coloured than carapace; chelal hand partly granulate, fixed and movable fingers of chela entirely smooth; chela with distinct pedicel; no venom ducts observable; coxa with acuminated setae; trochanter with a dorsal hump; femur with distinct pedicel (Fig. 4A); movable finger with 19-20 contiguous teeth; fixed finger with 21-23 contiguous teeth.

Trichobothriotaxy: fixed finger with eight and movable finger with four trichobothria; fixed finger with trichobothrium *et* situated close to tip of finger, *est* situated closer to *it* than to *et*, *it* situated slightly distal



FIGURE 4. Male *W. hispanus*. A) Left pedipalp, dorsal view; B) Left palpal chela with the trichobothrial pattern, lateral view; C) Tarsus IV, dorsal view.

to *ist*; movable finger with trichobothrium *st* approximately situated closer to t than to *sb* and *sb* situated close to *b* (Fig. 4B).

Legs: brown; distinctly lighter than body; not granulate; coxae with simple setae; claws almost stout and symmetric; arolia simple and shorter than claws (Fig. 4C).

Measurements (mm): Body length: 2.3-2.5; Carapace: 0.72-0.74/0.70-0.71; Pedipalp: trochanter 0.31-0.33/0.20-0.22; femur 0.60-0.64/0.22-0.24; patella 0.51-0.54/0.23-0.25; chela (with pedicel) 0.93-0.95/0.33-0.35; hand (without pedicel) 0.53-0.56/0.33-0.35; movable finger L. 0.38-0.41; Leg I: femur 0.14-0.16/0.12; patella 0.29-0.31/0.18-0.19; tibia 0.23-0.25/0.1; tarsus 0.23-0.24/0.06; Leg IV: femur+ patella 0.50-0.52/0.19-0.20; tibia 0.40-0.42/0.17-0.19; tarsus 0.30/0.09.

Genitalia (male): The lateral apodemes are triangular, long and very close together in the posterior region. Dorsal diverticulum and median diverticulum are approximately equal. Long ejaculatory canal extends to the abdomen (Figs. 5 A&D).

Female: not studied.

Distribution and habitat

W. hispanus is reported from southwestern Asia, many parts of Europe and northern Africa (World Pseudoscorpiones Catalog, 2024). It was recently reported from Slovakia (Christophoryová et al., 2012), Hungary (Novák, 2015), and Moldova (Červená et al., 2021). The current specimens represent the first record of this species from Iran (Fig. 1). The examined specimens were found under the bark of the alder tree in the area with humid temperate climate and high annual rainfall (Fig 6), but it can be found under the bark of plane trees (Kästner 1928; Červená et al., 2021), pine stumps, almond (Beier. 1966), elm (Beier 1963), locust, domestic apple trees (Zaragoza, 2007), and oak trees (Morano and Zaragoza, 2017; Hernández-Corral et al., 2018). Also, they can be found under stones (Dashdamirov, 1999).



FIGURE 5. Male genitalia of *W. hispanus*. A) Internal genitalia, ventral view; B) Male genitalia of *W. faunus* based on Heurtault (1971a); C) Male genitalia of *W. neglectus* (after Heurtault 1971a); D) Male genitalia of *W. hispanus*; E) Male genitalia of *W. hispanus* (after Heurtault 1971a).



FIGURE 6. habitat collected of *W. hispanus* under the bark of the alder tree.

Remark

Specimens of *Withius hispanus* from Iran show slight differentiation in the sizes of the pedipalps. The pedipalpal femur L/W ratio ranges from 2.66 to 2.72 mm, the chela (including the pedicel) L/W ratio ranges from 2.71 to 2.81 mm. In contrast, according to Novák (2015), the palpal femur L/W ratio for *W. hispanus* in Hungary is 2.74 mm, and the chela (including the pedicel) L/W ratio is 2.87 mm. Based on Christophoryová et al. (2012), the palpal femur L/W ratio for *Withius hispanus* in Slovakia is 2.90 mm, and the chela (including the pedicel) L/W ratio is 3.0 mm. Additionally, the small differences in the number of chelal finger teeth are minor between Iranian specimens and those from Hungary and Slovakia. In Hungary, the fixed finger has 19 contiguous teeth and the movable finger has 22, whereas in Slovakia, the fixed finger has 19 teeth and the movable finger has 21 contiguous teeth. These small differences are insufficient to justify the introduction of a different species and can be regarded as intraspecific variations within the species.

Withius hispanus differs from *W. nanus* in eastern northern Iran mainly by having chelicera galea with five minute rami, compared to four rami in *W. nanus* (see Nassirkhani & Hamidi 2015: Fig 2a). The trichobothrial pattern also differs between these two species (see Nassirkhani & Hamidi 2015: Fig 5). The

fixed finger of chela with 21-23 contiguous teeth and the movable finger with 19-20 contiguous teeth in *W. hispanus*, whereas the fixed finger with 21 cusped teeth and three external accessory teeth, and the movable finger with 26 cusped teeth and 2 external accessory teeth in *W. nanus*. These two species also differ based on some morphometric characters, such as the ratio of length to width (L/W) of the femur of the pedipalp (2.66-2.72) and the L/W ratio of the patella of the pedipalp (2.16-2.21) in *W. hispanus*, compared to the L/W ratio of the femur of the pedipalp (3.23) and the L/W ratio of the patella of the pedipalp (2.33) in *W. nanus* (Nassirkhani & Hamidi, 2015).

Nassirkhani and Hamidi (2015) did not refer to male genitalia in *W. nanus*, but *W. hispanus* can be easily distinguished from *W. nanus* based on male genitalia. *W. hispanus* has triangular and long lateral apodemes, whereas *W. nanus* has shortened lateral apodemes (see Mahnert 1988: Fig 45). *Withius hispanus* can be distinguished from *W. leggi* based on the difference in trichobothrial patterns: in *W. hispanus* fixed chelal finger trichobothrium *it* is situated in the level of the *ist* position; movable finger with trichobothrium *st* approximately situated closer to *t* than to *sb* (Fig. 4B). whereas in *W. leggi* fixed chelal finger trichobothrium *it* is situated in the level of the *isb* position; movable finger with trichobothrium *st* in the midway between *t* and *sb* (see Nassirkhani 2022: Fig 1J). These two species also differ in the number of teeth on the fixed and movable finger, with the fixed finger having 35 (36) teeth and the movable finger having 38 teeth in *W. leggi*. In terms of size, *W. hispanus* is smaller than *W. leggi*, with the femur of the pedipalp length 0.60-0.64 mm, the patella of the pedipalp length 0.51-0.54 mm, and the length of the chela (with pedicel) 0.93-0.95 mm in *W. hispanus*, compared to the femur and patella of the pedipalp length 0.78 mm and the length of the chela (with pedicel) 1.22 mm in *W. leggi* (Nassirkhani, 2022). *W. hispanus* and *W. leggi* can be separated from each other based on male genitalia, with *W. leggi* having shortened lateral apodemes (see Nassirkhani 2022: Fig 2 A&C).

Withius hispanus examined by Heurtault (1971a) can be separated from its congeners based on the structure of the male genitalia. According to the shape of the male genitalia, W. hispanus separated from species with shortened lateral apodemes, W. despaxi (Vachon, 1937; Harvey, 2015), and W. piger (see Heurtault 1971a: Fig 3). Withius faunus was considered as a synonym of W. hispanus by Beier (1932, 1963), although later these two species were separated based on key of the European and North African species which provided by Heurtault (1971b). The two species differ in the length/width ratio of the palpal femur (2.8 times in W. faunus, 2.66-2.72 times in W. hispanus) and the length/width ratio of the palpal patella (2.5 times in W. faunus, 2.16-2.21 times in W. hispanus) (Heurtault, 1971a, b). According to the study by Heurtault (1971a), the structures of the male genitalia in *W. faunus* (see Heurtault 1971a: Fig 6) is significantly different from W. hispanus. The chitinized arches in W. hispanus, lacking spicules and less concave. Median diverticulum is significantly longer than the dorsal diverticulum in W. faunus whereas they are approximately equal in W. hispanus. In W. neglectus (see Heurtault 1971a: Fig. 7) two chitinous arches are the same type as in W. faunus. The lateral apodeme run parallel in the distal third of their length, then diverge markedly and are interrupted at the level of the lateral apodem ends. The ventral diverticulum is similar to W. hispanus. The dorsal diverticulum is shorter than the median diverticulum, which is also the case in W. faunus. Little is known about the genitalia structure of W. japonicus and in the only illustration provided by Morikawa (1954), the male genitalia of W. hispanus is almost similar to W. japonicas (see Morikawa 1954: Fig. 1F). In male W. japonicus, palp femur is 3.1 times, patella 2.6 times and chela 2.9 times longer than wide (Morikawa, 1954).

DISCUSSION

The morphology of the male reproductive system has been described for various pseudoscorpion species (e.g., Vachon 1938; Legg 1974a, b, c, 1975a, b). Mahnert (1975) and Dashdamirov (1992) proposed an exhaustive investigation of the male and female genitalia is important to comprehend the classification within the Withidae. Despite the importance of male genitalia for understanding the phylogeny and classification of the Withidae (Romero-Ortiz & Sarmiento, 2021), only 13 out of 170 species belonging to six genera have been subjected to detailed morphological studies (Heurtault 1971a; Harvey 1988, 2004, 2015; Mahnert 1988; Dashdamirov 1992). This may be due to the difficulty in study of the reproductive

system in situ (Klausen, 2005) and that traditional characters may be lost through extraction of copulatory organs (Romero-Ortiz & Sarmiento, 2021).

Hreover, some species might have similar genitalia structures. The existing differences between the morphometric characters and chaetotaxic features could lead us to assigning individuals to different species (Heurtault, 1971a).

According to Harvey (2015), four species, including *Withius hispanus*, *W. faunus*, *W. neglectus*, and *W. japonicus*, should be transferred to another genus based on the morphological characteristics of their male genitalia structure. However, the number of species may increase as more illustrations and descriptions of the male genitalia structure are provided, alongside future research that incorporates morphological evaluations with molecular techniques.

Key to the species of the Withius of the Palaearctic realm

1. Internal trichobothrial series of the chelal fingers are basally clustered with trichobothria *it* and 2(1).isb situated approximately near to each other; Chela fingers equal the hand without pedicle; Eyes Internal trichobothrial series of the chelal fingers are slightly more widespread with *it* and *isb* separated 3(2). Fixed chelal finger with 21 teeth; Movable chelal finger with 26 teeth; Galea in chelicera with 4 rami; Serrula exterior with 16-18 blades; Palpal femur 2.80-3.0 times longer than wide..... Fixed chelal finger with 35 teeth; Movable chelal finger with 38 teeth; Galea in chelicera with 4 or 5 rami; Serrula exterior with 19 blades; Palpal femur 3.3 times longer than wide..... Fixed chelal finger with 28-31 teeth; Movable chelal finger with 31-35 teeth; Galea in chelicera with 3 or 5(4). Lateral apodeme in male genitalia run parallel in the distal third of their length; Palpal femur 3.1 Lateral apodeme in male genitalia are very close together in the posterior region and run parallel; Palpal 6(4). L/W palp femur 2.66-2.72 times longer than wide; L/W palpal patella 2.16-2.21 times longer than L/W palp femur 3.1 times longer than wide; L/W palpal patella 2.6 times longer than wide.....

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