First record of Amblypygi from Italy: *Charinus ioanniticus* (Charinidae)

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Abstract

The arachnid order Amblypygi is recorded for the first time in Italy, with the species *Charinus ioanniticus* (Kritscher, 1959). An isolated reproductive population was found in an underground air-raid shelter dating back to World War II below the city centre of Trieste. This represents the second record of this parthenogenetic species in continental Europe and also its westernmost known population.

Keywords: distribution • expansion • Kleine Berlin • parthenogenesis • Trieste • whip spiders

Introduction

Amblypygi is a pantropical order of Arachnida with around 220 described species divided into 5 families (Harvey 2003; Miranda & Zamani 2018). Whip spiders, as they are also called, are characterized by their flattened body, long first pair of legs (antenniform legs), raptorial pedipalps and two-segmented chelicerae. They are close relatives to Thelyphonida and Schizomida, with whom they share the antenniform first pair of legs, raptorial palps and muscle synapomorphies (Shultz 2007; Harvey 2013). Amblypygi are mostly nocturnal hunters that during daytime find shelter under rocks, in crevices or under tree bark (Weygoldt 2000).

This group of arachnids is widely distributed from the Americas to Africa, Eurasia, and Oceania; most of the diversity is found in tropical and subtropical areas of the Old and New World (Harvey 2003). Only three species are known to occur in the Western Palearctic: *Musicodamon atlanteus* Fage, 1939, in Morocco and Algeria, *Charinus ioanniticus* (Kritscher, 1959) in Egypt, Jordan, Israel, Turkey, and Greece (Rosin & Shulov 1960; Kovařík & Vlasta 1996; El-Hennawy 2002, 2019; Blick & Seiter 2016; Miranda *et al*. 2016a; Shakhatreh *et al*. 2020), and the recently described *Charinus israelensis* Miranda *et al*. 2016a endemic from Israel.

Charinus ioanniticus is the only species found in Europe, being recorded from the Greek islands of Rhodes and Kos. It has also recently been recorded for the first time in continental Europe in mainland Greece (Agapakis & Miranda 2019). *Charinus ioanniticus* is a synanthropic species that reproduces mostly by parthenogenesis (Weygoldt 2007; Blick & Seiter 2016; Miranda *et al*. 2016a; Agapakis & Miranda 2019) and, due to this peculiar reproduction style, the species can potentially establish disjunct populations after accidental transportation by humans once it finds a suitable habitat. This would explain the wide distribution range of *C. ioanniticus* across the eastern Mediterranean (Weygoldt 2005; Miranda *et al*. 2016a). However, it is still not completely clear where this species can be considered native and which of the populations are the result of accidental transportation.

In this work, we present the first record of a population of *Charinus ioanniticus* from Italy, found in an artificial gallery dating back to the Second World War (named Kleine Berlin), in the city centre of Trieste. Specimens have been observed many times, and were marked to count them more easily and to track their movements. Additionally, assuming this is an introduced population, a historical search for the potential original source of the first specimens brought to Italy was undertaken.

Materials and methods

The specimens were detected by simple inspection, hand collected, stored in 75% ethanol, and deposited at Museum of Natural History of Trieste, Trieste, Italy (MNHT). Photographs of the habitus and the habitat were obtained with an Olympus OM-D E-M1 camera equipped with a Zuiko ED 60 mm F2.8 Macro + Raynox DCR250 and an extension tube. Images of the examined specimens were taken with a Leica MC190HD camera or Olympus OM-D E-M1 camera mounted on a LEICA MZ16 stereomicroscope. Temperature and humidity in the tunnel were measured with an RC-51 Data Logger.

Five live specimens were marked *in situ* on the dorsal integument using an orange water marker pen for easier recognition and monitoring of the specimens (Fig. 1), fol-

Fig. 1: *Charinus ioanniticus* from Italy. One of the live specimens marked in orange for monitoring purposes.

lowing a harmless method frequently used for marking queen bees (Human *et al*. 2013). The taxonomic identification followed Miranda *et al*. (2016a).

Charinus ioanniticus (Kritscher, 1959)

New records: ITALY, Trieste, air-raid shelter Kleine Berlin, 45°39′19″N 13°46′40″E, 10–15 m, gallery in the direction of the city's Court of Justice; 1 juv., 08 January 2019, R. Bernardis leg. (MNHT); 1♀, 18 April 2019, C. M. Legittimo & A. Colla (MNHT); 1 exuvia, 30 January 2020, C. M. Legittimo & A. Colla (MNHT); 2♀♀, 2 exx. adults, 3 juv., April 2019/ August 2020, C. M. Legittimo & A. Colla (observed in the gallery, marked and photographed).

Morphology: All characters of the specimen from the Italian population match with the characters presented as diagnostic in *Charinus ioanniticus* (Miranda *et al*. 2016a) (Fig. 2A), such as: basitibia IV divided into four pseudo-articles; median eyes, tubercle and lateral eyes present and prominent; frontal border of carapace projected anteriorly and rhomboid; pedipalp femur with five dorsal and ventral spines; tibia I with 21 and tarsus I with 37 articles (Fig. 2B); finger like female gonopods. Considering the morphological evidence, the specimens present in the Kleine Berlin can be ascribed to *Charinus ioanniticus* (Kritscher, 1959).

Natural history and habitat: The new *Charinus ioanniticus* population was found in the Kleine Berlin German air-raid shelter in Trieste (Italy). The Kleine Berlin is an underground air-raid shelter built by the German occupants in 1944 in the central area of the city of Trieste. The galleries, dug under a flysch hill, are covered with thick, reinforced concrete walls. Since the end of the war, this complex of tunnels has remained virtually intact. Since 1996, the tunnel system has been entrusted to the Trieste Alpine Club (CAT), which has enhanced the structure and made it accessible mainly as an historical museum (Radacich 2010).

Inspections were carried out in all the galleries of the bomb shelter between February 2019 and August 2020 to assess the size of the population. Specimens were found in almost all the visits, for a total of nine recognized specimens (juveniles with body length of 4–5 mm and adults with body length between 6–9 mm) and numerous exuviae of different size. All marked individuals were recurrently observed and photographed (Fig. 3). One adult-sized specimen was collected for morphological analysis and future genetic analysis.

All the individuals were found in a single branch of the underground complex, which is the main tunnel gallery that in the past led to the city's Court (Figs. 4–5). This section is always closed to visitors and separated from other tunnels by a metal door, thus being isolated from the external environment. It extends for a total of about 80 m, with a cross section of 2.25 m × 2.5 m in height (Guglia, Halupca & Halupca 2001), is generally not illuminated, and shows similar characteristics of a natural hypogean cavity.

The concrete walls are always damp, with constant presence of condensed water. In many sections, the infiltration of water coming from the hills above causes the formation of large calcareous milky concretions. The metal and wooden artifacts, dating back to the 1940s, are now completely corroded by rust or rotten. The concrete floor in some areas is submerged by water.

Temperature and relative humidity were 16.8°C, 82% HR (05 April 5 2019), 16.9°C, 96% HR (04 December 4

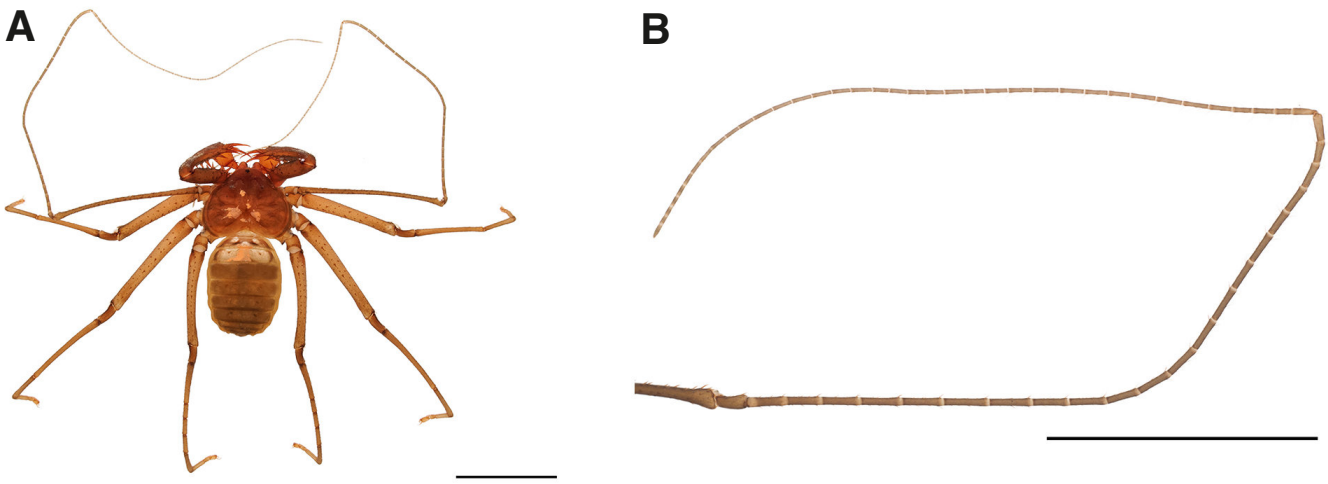

Fig. 2: *Charinus ioanniticus*. **A** female stored in 75% ethanol; **B** tibia I with 21 and tarsus I with 37 articles. Scale bars = 5 mm.

Fig. 3: *Charinus ioanniticus* on the concrete wall of the gallery.

Fig. 4: The tunnel in Kleine Berlin air-raid shelter where *Charinus ioanniticus* occurs.

2019), 14.8°C, 87% HR (30 January 30 2020) and 17.5°C, 95% HR (24 May 24 2020). Due to the absence of ventilation and openings towards the outside, thermo-hygrometric conditions seem to be constant throughout the year. This is confirmed by the guardians of the anti-aircraft complex who assess that minimal fluctuations occur between summer and winter. A thermo-hygrometer Data Logger was installed in the gallery to evaluate with more precision the environmental parameters in the future.

The place where *Charinus ioanniticus* inhabits is not completely free of pollutants. In some section, the walls of the tunnel are impregnated with oily compounds and fuel due to spills, dating back to the 1940s and 1950s. Moreover, the seeping waters are likely to bring pollutants from the overlying busy roads. However, chemical and microbiological analyses should be carried on to investigate better these aspects.

The arthropods present in the branch of the complex of galleries with the *Charinus* population show a relatively low diversity, represented mainly by Diplopoda (*Callipus foetidissimus* (Savi, 1819)) and small Araneae (*Kryptonesticus eremita* (Simon, 1880)). Moreover, numerous Collembola, Annelida, Isopoda, and Acari can be found near pieces of rotting wood. Among the Amphipoda, a single specimen of the family Talitridae has been collected; the crustacean was jumping on the soil of the gallery, near the water, just below the walls frequented by *C. ioanniticus*. Given the presence of numerous fractures in the concrete walls, the

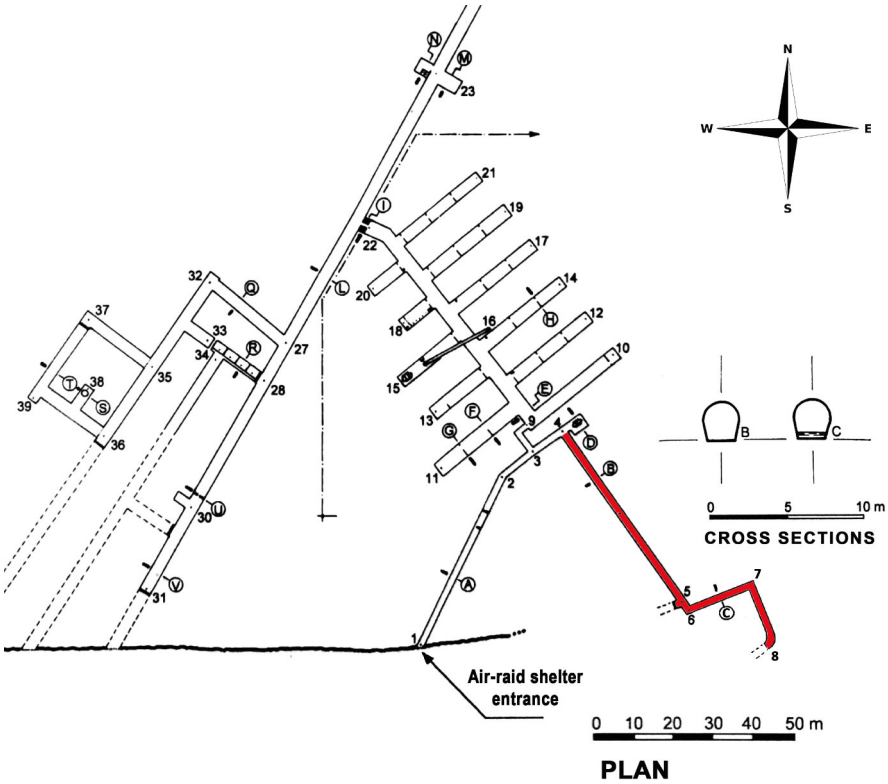

Fig. 5: Kleine Berlin air-raid shelter; plan and cross sections. The tunnel where *Charinus ioanniticus* occurs is marked in red. Modified from Guglia, Halupca & Halupca (2001).

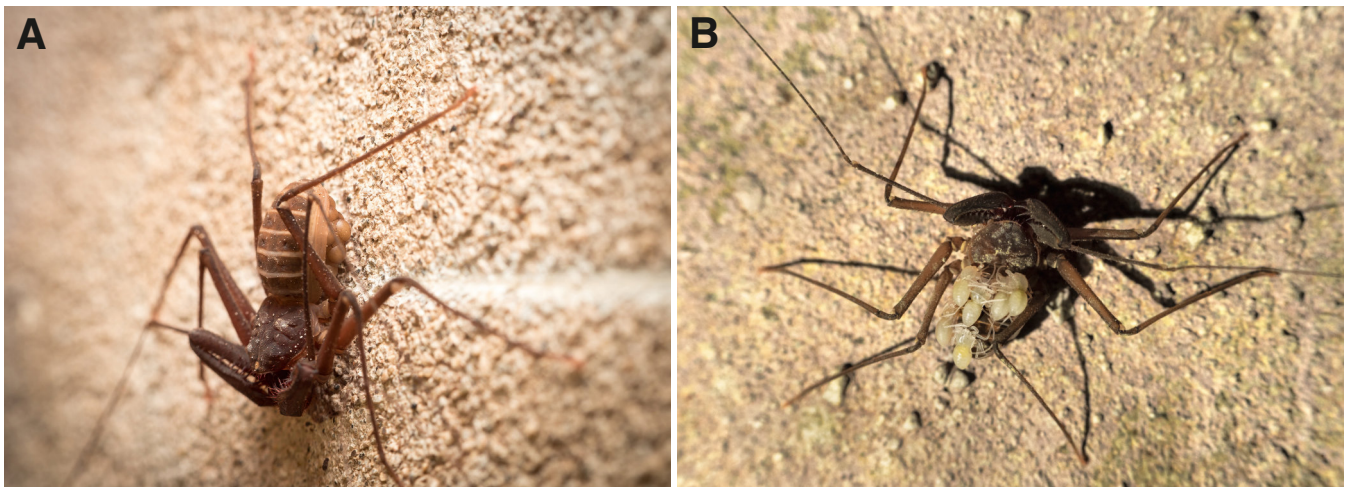

Fig. 6: *Charinus ioanniticus*. **A** female with eggs (6 July 2020); **B** female with praenymphae (1 October 2020).

gallery is also in connection with the natural hypogean habitats of the hill.

The specimens of *C. ioanniticus* were always vertically positioned on the walls and never on the floor. They were often seen walking or standing on calcareous concretions and were frequently covered with water droplets. It was also noted that several specimens stayed in the same portion of the wall, as if they had some kind of territorial habits. It has been observed in *Charinus ruschii* Miranda *et al*., 2016b that the small, young specimens prefer to live holed up in fractures (Miranda *et al*. 2016b). This could also be possible for *Charinus ioanniticus* and, therefore, it cannot be excluded that more specimens are present in the gallery. During June–July 2020, two of the large specimens laid eggs. They were observed and photographed several times during the long brooding and egg development. Praenymphae were observed on the mother's backs in the first days of October 2020 (Fig. 6). Compared to previous observations, these specimens seemed to be more timid, prefering to stay in more sheltered areas and near fractures in the concrete. If disturbed, they immediately sought shelter.

On the vault of the gallery, numerous exuviae of different sizes were identified (Fig. 7). For ease of recognition, these were circled with a white chalk mark in order to count them in the future. It is plausible that the exuviae will soon be destroyed, due to humidity and the presence of detritivorous Diplopoda on the vault.

Discussion

This is the first report for an Amblypygi population in the Italian territory. Trieste is completely out of the known range of this species and the discovery is therefore peculiar.

To date, the presence of *C. ioanniticus* was known for Europe only by observations on the Greek mainland (Agapakis & Miranda 2019) and Greek islands not far from Turkey (Blick & Seiter 2016) (Fig. 8).

Most of the previous reports about this species describe populations composed exclusively by female individuals (Weygoldt 2007), apart from Kovařík & Vlasta (1996) and Weygoldt (2005) who reported the presence of males in Turkish populations. No males have been found yet in the Italian population. *C. ioanniticus* is usually observed in the underground galleries and ruderal urban buildings. The conditions observed in Trieste are thus comparable with what is known from the bibliography. In particular, analogies can be found with a report from Turkey (Kovařík & Vlasta 1996) where the specimens were observed in a karst cavity with concretions and water infiltrations from fractures in the walls, and with the presence of this species in the sewerage system of Jerusalem (Miranda *et al*. 2016a).

Hypotheses for the origin of the population

Given its known range, it is unlikely that the new population of *Charinus ioanniticus* is native to Italy. The closest known population is the one from continental Greece, distant around 1200 km. Speleological research in the Trieste territory and the Dinaric Karst has been very active for over 100 years and the presence of these particular arachnids would not have gone unnoticed.

Fig. 7: Small exuvia of *Charinus ioanniticus* on the vault of the gallery.

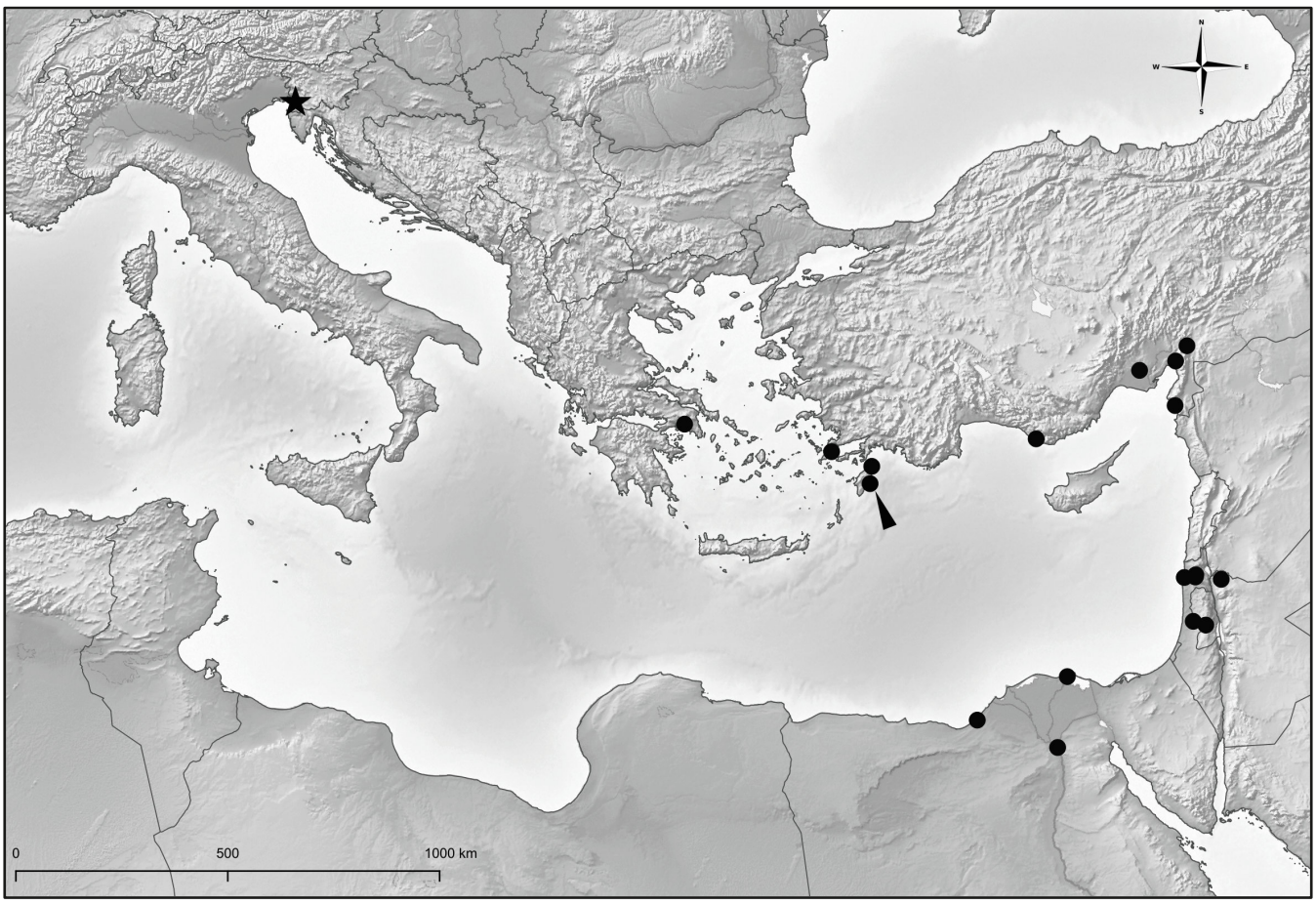

Fig. 8: Map of the current distribution of *Charinus ioanniticus*. Star indicates population in Trieste, Italy; arrowhead indicates the type locality; black circles indicate previous records.

Fig. 9: *Charinus ioanniticus*. Habitus of some Italian specimens.

Country	Expedition	Date	Activities	Localities	Elevation (m)	Coordinates	Datum
Greece	International Congress of Speleology	August 2005	Archaeological visits	Athens, Kalamos and anthropic cavities in Delphi	–	–	–
	Expedition to the western shore of the Mani Peninsula		Speleosub exploration	Vatsinidi cave (Proastio), 3 km NW of Stoupa	Sea level	36°52′10.6180″N 22°14′46.6662″E	ED 1950
				Selinitsa-Dracos Cave System	Sea level	36°48′10″N 22°17′50″E	WGS84
			Speleological exploration	"Cave W of Achilio"	87	36°26′14.95″N 22°29′06.65″E	ED 1950
				"Cave SW of Kyparissis"	Sea level	36°27′19.50″N 22°27′05.12″E	ED 1950
				"Small cave at Limeni Bay (Areopolis)"	10	36°27′19.11″N 22°27′37.25″E	ED 1950
				Varatro Janes Tomers cave (Thalames)	470	36°47′06″N 22°20′04″E	WGS84
				Katafygi cave (Dimistios)	Sea level	36°48′10″N 22°17′50″E	WGS84
				Vatsinidi cave (Proastio), 3 km NW of Stoupa	20	36°52′10,6180″N 22°14′46.6662″E	ED 1950
				Cave close to Kalianeika (NW of Kardamyli), entrance by the small St Nikolaos church	340	36°55′25″N 22°09′44″E	WGS84
			Speleo-archeological visits to anthropic cavities	Mycenae, artificial cavities: water well, square section tunnel, and water collection tank	278	–	–
				Akrokorinthos, Artificial cavities (tunnels and reservoirs)	575	–	–
	Expedition to the uplands of Aygherinos and Vikos (Zagoria regiojn, Pindus Mountains)	August 2011	Speleological exploration	Vikos–Aoös National Park, 42 caves explored and mapped	1200–1600	39°58′10″N 20°43′41″E	WGS84

Table 1: Explorations made by the Speleological group of CAT in Greece. Source: CAT research diaries.

In many years of visits to the shelter, the operators of the Trieste Alpine Club (CAT) have never observed this arachnid before February 2019. However, the discovery of several specimens of different sizes and exuviae on the gallery's vault, as well as the discovery of two large females with eggs and praenymphae, suggests that the population has adapted to the place and is reproductive there. With the available information, it is not possible to estimate when the first specimen/specimens arrived, however, some hypothesis about the mode of arrival of the Amblypygi are made.

Hypothesis 1: the specimens could have been introduced in the Kleine Berlin by speleological material or historical objects that have been stored in the galleries over the years. Despite interviews with all the most active members of the speleological group of CAT and analysing the journals of their expeditions in the Near East, we could not find reliable links between the speleological activities and an accidental transportation of Amblypygi in Italy.

All speleological activities performed by CAT in areas where *C. ioanniticus* could be present are provided in Table 1. These indications may be useful to investigate the presence of *C. ioanniticus* in the Greek caves explored by CAT and listed here.

Regarding Turkey, the latest expeditions date up to more than 35 years ago, when CAT was not already managing Kleine Berlin.

Hypothesis 2: The specimens could have been accidentally transported to Trieste with the numerous trades by ship with Greece, Turkey and Aegean Islands. The specimens could have migrated from the large port areas to the city center and the Kleine Berlin through large urban areas. Perhaps this was possible through the numerous sewer systems and artificial cavities present under the city. In this case, it might be possible that other populations of *Charinus ioanniticus* inhabit other artificial tunnels with similar characteristics. However, most of these tunnels are far away from the new commercial harbour and were already well explored by the Adriatic Speleological Society, which did not find amblypygids yet (Guglia, Halupca & Halupca 2001).

Further investigation will be needed to elucidate the presence of these Amblypygi in Trieste. The discovery of this new population and the availability of several live specimens in the gallery opens possibilities for more research about the biology, ecology and adaptation capabilities of *C. ioanniticus*.

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