Received: 24 June 2020 | Revised: 23 December 2020 | Accepted: 5 January 2021

DOI: 10.1111/jzs.12457

JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONARY RESEARCH — WILEY

ORIGINAL ARTICLE

Range-wide phylogeography and taxonomy of the marine rock pools dweller *Tigriopus fulvus* (Fischer, 1860) (Copepoda, Harpacticoida)

Luca Vecchioni[1] | Marco Arculeo[1] | Vezio Cottarelli[2] | Federico Marrone[1]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy

[2]Department for Innovation in Biological, Agro-Food and Forest Systems, Tuscia University, Viterbo, Italy

Correspondence
Luca Vecchioni. University of Palermo, Via Archirafi 18, Palermo 90123, Italy.
Email: luca.vecchioni@unipa.it

Abstract

In the light of the wide distribution and ecological importance of the genus *Tigriopus* in coastal rock pool habitats, and of its frequent use in aquaculture and as a model organism, we investigated the identity of the Atlantic–Mediterranean *Tigriopus* populations and elucidated their taxonomy and patterns of morphological and genetic diversity. In order to reach these goals, an "integrative taxonomy" multisource approach was implemented. First, we investigated the constancy and taxonomical value of the morphological characters currently used to distinguish among the *Tigriopus* species occurring in the Mediterranean and in Eastern Atlantic area north of the Tropic of Cancer, and checked the actual morphological differences possibly present among topotypical samples of *Tigriopus fulvus fulvus* (Fischer, 1860) and its two subspecies *Tigriopus fulvus adriaticus* Van Douwe, 1913 and *Tigriopus fulvus algiricus* Monard, 1935. Then, we sequenced fragments of mitochondrial (*12S*) and nuclear (*28S*) genes. In the frame of this study, different "DNA taxonomy" approaches were implemented in order to check whether the subspecies of *Tigriopus fulvus* were actually lineages evolving independently, that is, valid species according to the "evolutionary genetic species concept." The results coherently indicate the presence of a single species, characterized by constant morphology and a noteworthy geographically based genetic structure in the whole study area. No morphological or genetic support was found for the taxa of allegedly subspecific rank within *T. fulvus*, which are thus to be considered junior synonyms of *T. fulvus* s.s. Finally, a restricted *locus typicus* is established for *T. fulvus*, and a neotype is designated.

KEYWORDS
DNA taxonomy, genetic structuring, Harpacticidae, rocky shore communities

1 | INTRODUCTION

Marine rock pools are extreme habitats mainly populated by diatoms, flagellates, and a few specialized animal taxa such as rotifers, crustaceans (mostly harpacticoid copepods), mollusks and insects (mostly hydraenid beetles and culicid flies) (Antonini et al., 2010; Audisio et al., 2010; Issel, 1914; Mastrantonio et al., 2015; Vecchioni et al., 2019). These habitats are very dynamic and subject to pronounced daily and seasonal chemical and physical variations (Ganning & Wulff, 1970; McAllen, 1999; Underwood & Skilleter, 1996); during the year, and even during a single day, considerable variations in temperature, salinity, amount of water, pH, and oxygen

content occur (Powlik, 1999). These extreme conditions can cause high mortality in most of the resident organisms, leading to their recurrent local extinctions, followed by the recolonization of the rock pools. Accordingly, rock pools dwellers have developed mechanisms that allow them to escape or face the adverse conditions mentioned above. Organisms capable of flying, like some beetles or mosquitoes (Antonini et al., 2010; Mastrantonio et al., 2015), can actively move between pools during periods of drought, whereas other taxa produce resistance stages to overcome, *in situ*, the adverse periods (Williams, 2006).

Tigriopus Norman, 1869 is a harpacticoid genus typically related to the rock pools occurring in the supratidal and the uppermost intertidal zones (Vecchioni et al., 2019 and reference therein). This genus is present with active stages throughout the year, whenever water is available, with some exceptions linked to extreme temperatures (Issel, 1914; and personal observations). The adult *Tigriopus* are able to enter in a state of quiescence to survive moderately adverse conditions, and the conditions in which the phenomenon occurs have been the object of several studies (e.g., Issel, 1914; Powlik & Lewis, 1996; Ranade, 1957; Vittor, 1971). The transition to the state of quiescence takes place gradually, with an initial loss of vivacity, which ends with a state of complete immobility. When adverse conditions end, the copepods resume their normal activity. In addition to the quiescence stage as a resistance mechanism, *Tigriopus* can also adopt different behavioral adaptations that allow facing short-lasting desiccation. Both the larval stages and the adults, which are able to dig in the mud, can find a humid refuge in the sediment when it is present at the bottom of the rock pools (McAllen, 1999). McAllen (1999) also reported the finding of several hundreds of adult and immature specimens of *T. brevicornis* (Müller, 1776) within the cavity of a single thallus of *Ulva intestinalis*, a green alga sometimes occurring in the rock pools inhabited by *Tigriopus brevicornis* (Davenport et al., 1997; Handschumacher et al., 2010). The internal cavity of the algae can in fact provide a moist and hydrated environment adequate to protect *Tigriopus* spp. from dehydration for days and even weeks (e.g., Powlik & Lewis, 1996).

To date, the passive dispersal mechanisms of the genus *Tigriopus* are unknown, but several hypotheses have been proposed. Davenport et al. (1997) and Handschumacher et al. (2010) suggested that groups of floating algae, such as uprooted *Ulva* thalli, could act as means of transport for hypothetical colonies of copepods present within them. However, Powlik (1999) noted that the presence of *Tigriopus* appears to be independent of the presence of algae or marine plants in the rock pools, which are generally poor in macroflora, as we can also confirm based on our personal observations. Rock pools are occasionally frequented by birds, that are known to be able to disperse organisms for long distances, transporting their resting stages through endo- and epizoochory (Incagnone et al., 2015); although no cases of *Tigriopus* transport have yet been documented through avifauna, other microcrustaceans have been routinely found in bird plumage (Incagnone et al., 2015; Powlik, 1999; Swanson, 1984). Anemochory and hydrochory are other candidate dispersal modes, as wind or sea currents and tides can transport

TABLE 1 Origin of the studied *Tigriopus* samples. Geographic coordinates are expressed as decimal degrees (Map Datum: WGS84)

Locality code	Geographic origin	Latitude (N)	Longitude (E)
Tigriopus fulvus			
TIP	Algeria, Tipaza[a]	36.6229	02.4081
ROV	Croatia, Rovinj[b]	45.1172	13.6071
TER	Italy, Terrasini	38.1542	13.0756
LIN	Italy, Linosa	35.8632	12.8547
GRA	Italy, Torretta Granitola	37.6067	12.6257
BAR	Italy, Barcarello	38.2129	13.2916
MAG	Italy, Magnisi	37.1562	15.2369
PLE	Italy, Plemmirio	37.0021	15.3315
MIL	Italy, Milazzo	38.2700	15.2245
COR	Italy, Cornino	38.0900	12.6583
UST	Italy, Ustica	38.7089	13.1963
PAN1	Italy, Pantelleria	36.7793	11.9541
PAN2	Italy, Pantelleria	36.8158	11.9263
POR	Italy, Portoscuso	39.2065	8.3762
TRI	Italy, Tricase	39.9330	18.3975
CEF	Italy, Cefalù	38.0415	14.0218
CAS	Italy, Castiglioncello	43.4012	10.4045
GAR	Italy, Gargano	41.9264	15.6435
USA	Italy, Usai	39.1096	9.5231
GEN	Italy, Genova Nervi	44.3826	9.0261
KOK	Greece, Kokkinoreia	36.4019	22.4873
SDI	Morocco, Sidi Ifni	29.3467	−10.1961
CPT	Morocco, Cape Tamri	30.5465	−9.7180
SXL	Portugal, Seixal (Farrobo)[c]	32.8270	−17.1145
PDC	Portugal, Porto da Cruz[c]	32.7763	−16.8264
JAV	Spain, Jàvea	38.7635	0.2050
BEN	Spain, Benitachell	38.7080	0.1664
MEN	Spain, Menorca	39.9980	3.8274
AKA	Cyprus, Ammos tou Kambouri	34.9785	34.0233
FKP	Cyprus, Faros—Kato Paphos	34.7609	32.4030
YEO	Cyprus, Agios Georgios	34.9026	32.3170
BIZ	Tunisia, Bizerte	37.3341	9.8408
T. brevicornis			
GLC	Spain, Sanxenxo	42.3898	−8.7767
TRD	Norway, Trondheim	63.4502	10.4323
T. californicus			
TCL	–	–	–

[a]Type locality of *Tigriopus fulvus algiricus*.
[b]Type locality of *Tigriopus fulvus adriaticus*
[c]*terra typica* of *Tigriopus fulvus* s.s.

organisms from one rock pool to another (Incagnone et al., 2015; Powlik, 1999), but no such evidences have to date been collected for the genus *Tigriopus*. Finally, humans could also be possible dispersal vectors, considering the widespread use of *Tigriopus* spp. in aquaculture as live food for fish and invertebrates, although to date no ascertained cases of human-mediated introductions of the genus *Tigriopus* are reported (Handschumacher et al., 2010).

Over the years, the systematics of the genus *Tigriopus* has undergone some changes, mostly concerning the species *T. brevicornis* and *T. fulvus*. In fact, the systematics of the genus is still partly unsettled and incomplete or contrasting information about its taxonomy and distribution is present in the currently available synopses (e.g., Boxshall & Defaye, 2020; Dussart & Defaye, 1990; Walter & Boxshall, 2020; Wells, 2007). Müller (1776), based on specimens collected along the Norwegian coasts (Strøm, 1765), described the copepod *Cyclops brevicornis*. Nearly a century later, Fischer (1860) described a harpacticoid copepod, native of Madeira, which he named *Harpacticus fulvus*. A few years later, Norman (1869), based on samples collected in England, described *Tigriopus lilljeborgii*, which was later considered a junior synonym of *H. fulvus* by Brady (1872) and Sars (1911). Moreover, the latter author, in his study on Norwegian harpacticoids, reviewed the genus *Tigriopus* recognizing *Tigriopus fulvus* as the only valid species of the genus, thus putting all the other taxa as synonyms of *T. fulvus*. However, according to the Principle of Priority (art. 23) of the International Code of Zoological Nomenclature (ICZN, 1999 - www.ICZN.org) *Tigriopus fulvus* should have rather been considered a junior synonym of *T. brevicornis*. The reader should here note that the drawings made by Sars (1911) under the binomen *Tigriopus fulvus* in fact represented *Tigriopus brevicornis* specimens from Norway, as also evident through the comparisons of these drawings with those published by Fischer (1860) and Müller (1776). As a consequence, the two "varieties" of *T. fulvus* later described by Van Douwe (1913) and Monard (1935) were in fact established through a comparison with a different species, that is, *Tigriopus brevicornis*. In fact, using the description of Sars (see plate XXXI-XXXII, 1911) as a comparison, Van Douwe (1913) described an Adriatic "variety" of *Tigriopus fulvus* (*T. fulvus* var. *adriatica*), based on animals collected in Rovinj, Croatia. Monard (1935), based on the same description by Sars (1911), described *T. fulvus* var. *algirica*, from "Tipaza" (now Tipasa), in Algeria. Considering the species described by Müller (1776) and Fischer (1860) as synonyms and following the principle of priority of the ICZN, Lang (1948) established the priority of the binomen *Tigriopus brevicornis* (Müller, 1776), thus placing *T. fulvus* in synonymy with it, and maintaining the varieties described by Van Douwe (1913) and Monard (1935) as varieties of *T. brevicornis*.

Božić (1960), studying the morphology of the southern and northern European *Tigriopus* populations, and performing hybridization experiments, established that they actually belonged to two different species: *T. brevicornis* (Müller, 1776) and *T. fulvus* (Fischer, 1860), corresponding, respectively, the former to the northern Atlantic European populations, and the latter to those occurring in the Mediterranean Sea, in Madeira and part of the Atlantic coasts at the same latitudes (see Carli & Fiori, 1977). According to the Article

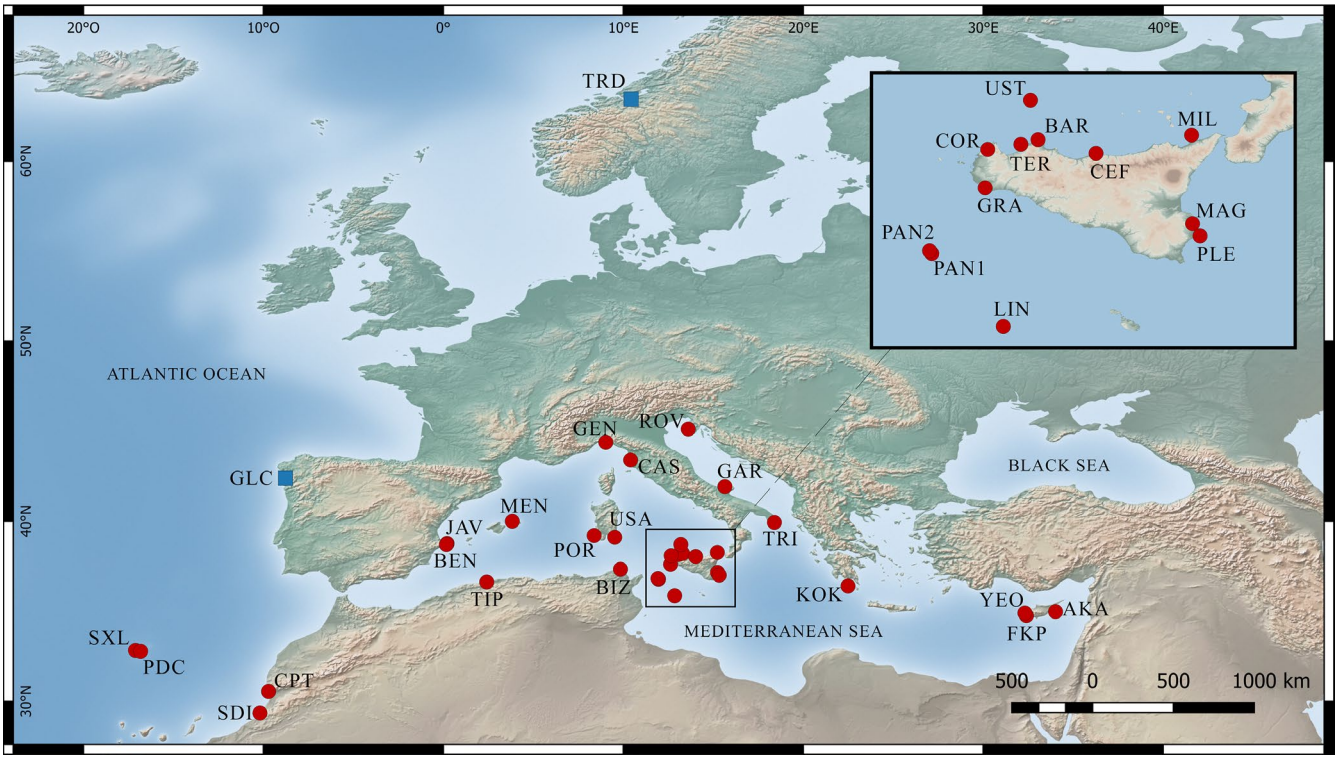

FIGURE 1 Geographic location of the sampled sites. Circles indicate those sites where *Tigriopus fulvus* was sampled; squares indicate occurrence sites for *T. brevicornis*. See Table 1 for the coordinates and codes of the sampled sites

45.6.4 of the ICZN (1999), if a taxon of infrasubspecific rank was established before 1961, it is currently to be considered as a subspecies. Accordingly, as already pointed out by Vecchioni et al. (2019), the two varieties mentioned above are nowadays to be considered subspecies of *T. fulvus* s.s. (sensu Božić, 1960). *T. fulvus* is therefore currently considered a polytypic species, and includes the subspecies *T. fulvus fulvus* (Fischer, 1860) from Madeira, *T. fulvus adriaticus* Van Douwe, 1913 from Rovinj, and *T. fulvus algiricus* Monard, 1935 from Tipasa.

In the Atlantic–Mediterranean area (i.e., the Mediterranean Sea and the Eastern Atlantic Ocean south to the Tropic of Cancer), three *Tigriopus* species are to date allegedly reported to occur: *Tigriopus fulvus*, *T. brevicornis*, and *T. brachydactylus* Candeias, 1959. However, *T. brachydactylus* was actually erroneously reported to occur in Northern Europe by Chullasorn et al. (2011) and Chullasorn et al. (2013), whereas, as already evidenced by Park et al. (2014), the species was originally described from Angola (western coast of Southern Africa) and never reported for European coasts. Moreover, *T. minutus* Božić, 1960 was described just south of our study area, and reported to occur in Senegal (Božić, 1960).

To date, despite the frequent use of *T. fulvus* and other species of the genus in aquaculture and ecotoxicology (e.g., Biandolino et al., 2018), the knowledge on the actual diversity of the genus *Tigriopus* in the Atlantic–Mediterranean area is limited and needs to be further investigated. The purpose of this study was thus to explore the morphological and genetic diversity of *T. fulvus* and its alleged subspecies throughout their distribution ranges, and to elucidate their taxonomy and patterns of morphological and genetic diversity based on an integrative approach, implementing the necessary formal taxonomical acts when opportune. In order to reach these goals, the exploratory mtDNA-only data provided by Vecchioni et al. (2019) are here expanded through new analyses based on an increased sampling effort, the sequencing of additional nuclear and mitochondrial molecular genetic markers, and

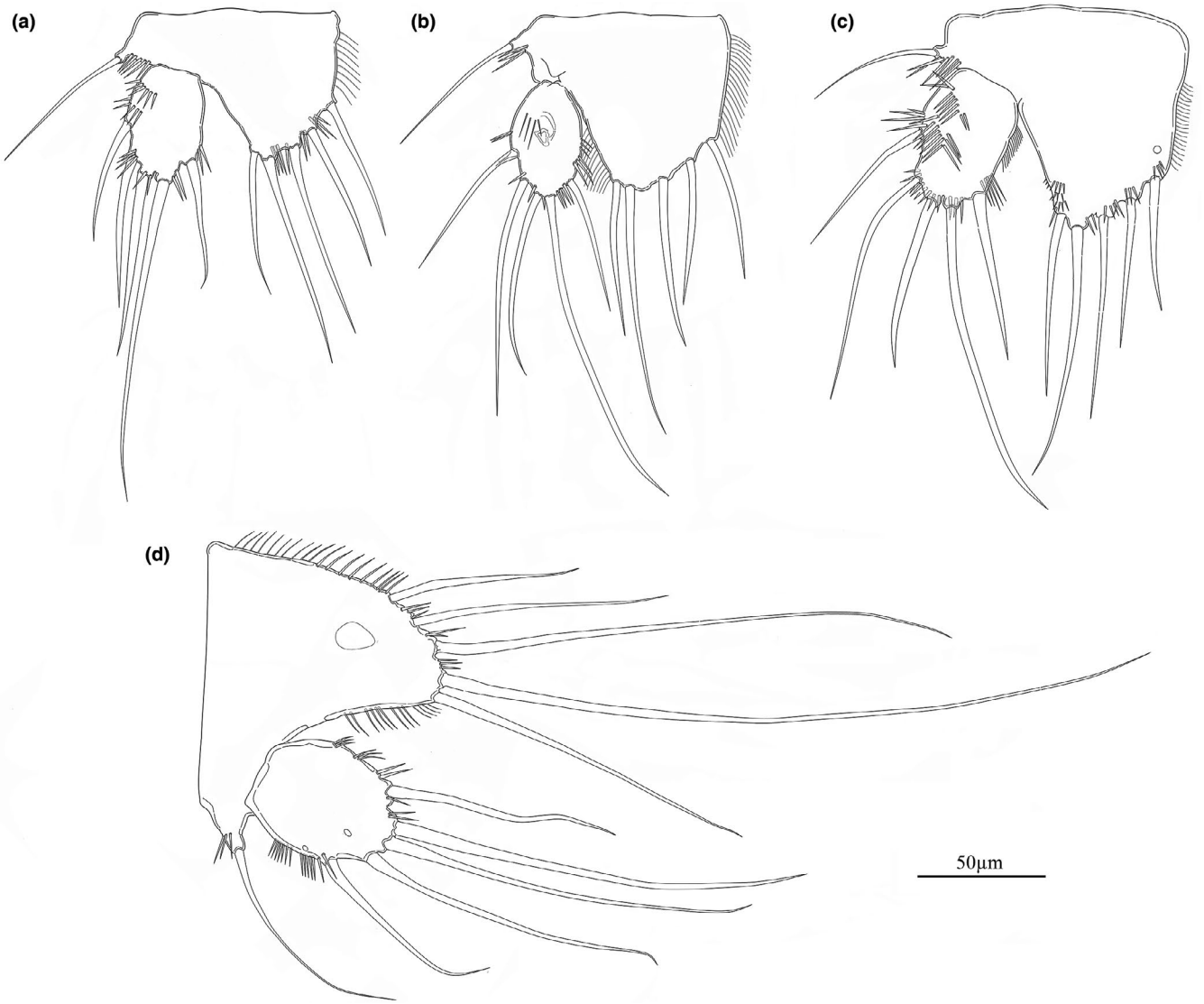

FIGURE 2 Female. a: fifth pair of legs (P5) of *Tigriopus fulvus adriaticus* (Rovinj, Croatia—ROV); b: P5 of *T. fulvus* s.s. (Seixal, Madeira, Portugal—SXL); c: P5 of *T. fulvus algiricus* (Tipaza, Algeria—TIP); d: P5 of *T. brevicornis* (Sanxenxo, Spain—GLC)

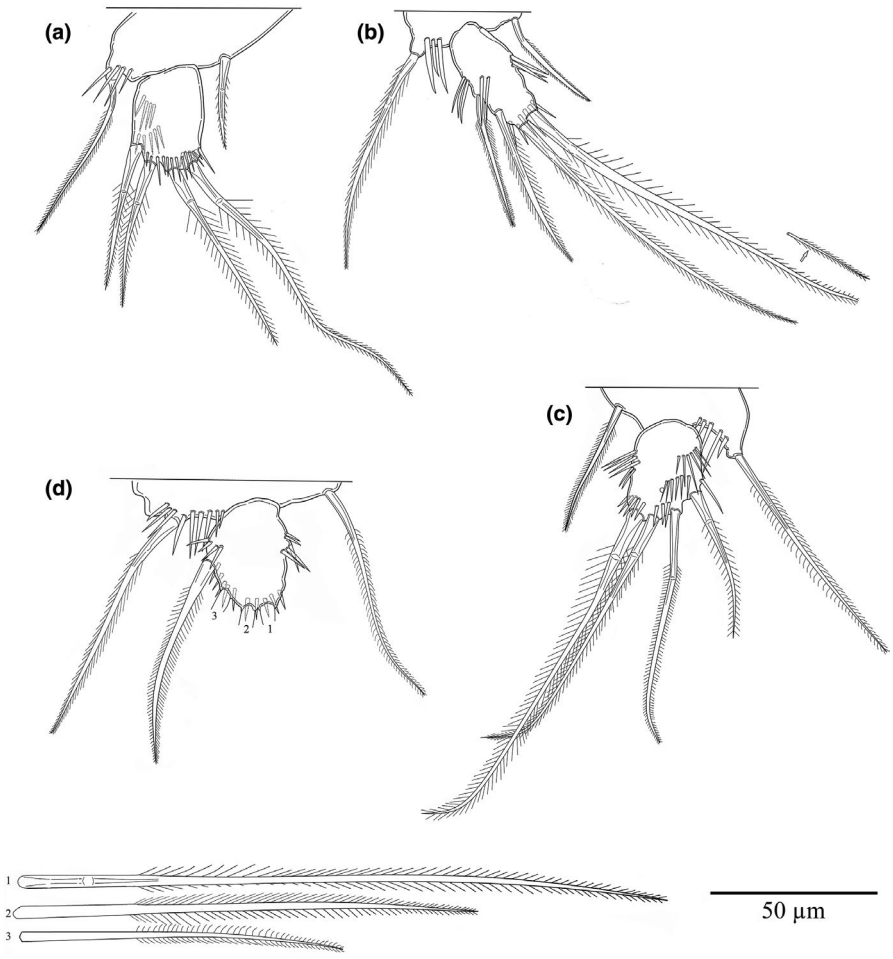

FIGURE 3 Male. a: fifth pair of legs (P5) of *Tigriopus fulvus adriaticus* (Rovinj, Croatia—ROV); b: P5 of *T. fulvus* s.s. (Seixal, Madeira, Portugal—SXL); c: P5 of *T. fulvus algiricus* (Tipaza, Algeria—TIP); d: P5 of *T. brevicornis* (Sanxenxo, Spain—GLC)

the morphological analysis and characterization of topotypical Mediterranean *Tigriopus* populations.

2 | MATERIALS AND METHODS

2.1 | Sampling and morphological identification

Specimens of *Tigriopus* spp. were collected from 2016 to 2019 in supratidal and intertidal rock pools from 33 sites located along the coasts of the Mediterranean Sea and the eastern Atlantic Ocean (Table 1 and Figure 1). In addition, a single sequence of *T. californicus* of unknown origin was obtained from a commercial strain of the species kindly provided us by D. Abed-Navandi (University of Vienna, Austria). *Tigriopus californicus* and *T. brevicornis* were used as out-groups in the phylogenetic analyses. The sampling sites were geolocated by GPS. The map of the sampling sites was created using the QGIS (2016) software v.2.18.2 (http://www.qgis.org).

Harpacticoids were sampled with a 200-µm mesh-sized hand net or a sieve with the same mesh size for the shallower pools where no nets could be used. Collected specimens were fixed *in situ* in 96% ethanol and sorted out in the laboratory under a stereomicroscope. For the morphological identification of the collected harpacticoids, the identification keys of Wells (2007) and the original descriptions of the species (Božić, 1960; Carli & Fiori, 1977; Fischer, 1860; Müller, 1776) and subspecies (Monard, 1935; Van Douwe, 1913) were used. For each site, at least five male and five females (350 individuals in total) were dissected in order to identify them and to check the validity and constancy of the diagnostic morphological characters. Specimens were rinsed in distilled water, dissected, and mounted in Faure's solution or lactic acid between two cover slips to allow observations from both sides. Illustrations were made at different magnifications up to a maximum of 1,250×, using drawing tubes mounted on a Zeiss Axioskop® phase-contrast microscope and a Polyvar Reichert-Jung® interferential-contrast microscope. Dissected specimens are currently stored in LV's collection at the Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche of the University of Palermo, Italy, and are available for loan on request. Moreover, individuals of *T. fulvus* s.l. from the populations of Madeira (one female and one male), Rovinj (one female and one male) and Tipasa (one female and one male), that is, from the *loci typici* of *T. fulvus* s.s. (Madeira, Portugal) and the alleged subspecies *T. fulvus adriaticus* (Rovinj, Croatia) and *T. f. algiricus* (Tipasa, Algeria), and two individuals of *T. brevicornis* from Galicia (Sanxenxo, Spain) (one female and one male) were drawn (see Figures 2-5).

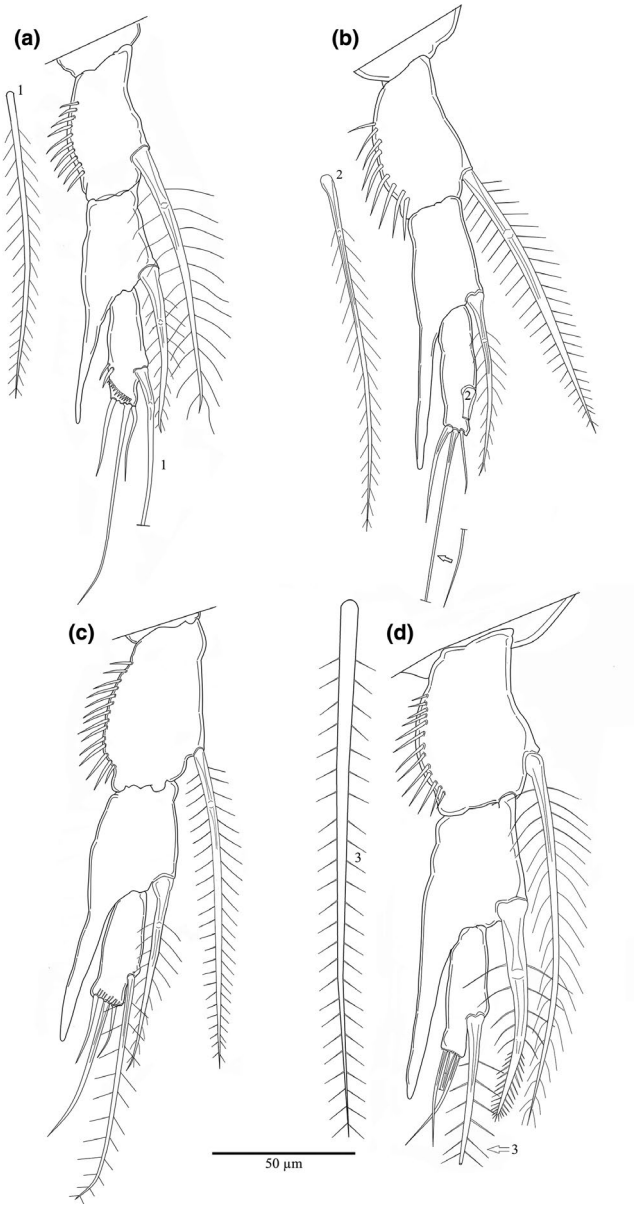

FIGURE 4 Male. a: Endopodite of the second pair of legs (P2) *Tigriopus fulvus adriaticus* (Rovinj, Croatia—ROV); b: P2 endopodite of *T. fulvus* s.s. (Seixal, Madeira, Portugal—SXL); c: P2 endopodite of *T. fulvus algiricus* (Tipaza, Algeria—TIP); d: P2 endopodite of *T. brevicornis* (Sanxenxo, Spain—GLC)

Tigriopus spp. voucher specimens (26 specimens in total) were deposited in the collection of the Zoology Section "La Specola," Natural History Museum, University of Florence (Italy) with the collection numbers (MZUF): 647–650 (*T. fulvus* from Madeira, "SXL"); 651 (*T. fulvus* from Cyprus, "FKP"); 652 (*T. fulvus* from Tyrrhenian peninsular Italy, "GEN"); 653, 657 (*T. fulvus* from Sicily, "LIN" and "BAR"); 654 (*T. fulvus* from Greece, "KOK"); 655 (*T. fulvus* from Croatia, "ROV"); 656 (*T. fulvus* from Tunisia, "BIZ"); 658 (*T. fulvus* from Algeria, "TIP"); 659 (*T. fulvus* from Atlantic coast of Morocco, "CPT"); and 660 (*T. brevicornis* from Norway, "TRD").

2.2 | DNA extraction, amplification, and molecular genetic analyses

One to three specimens (81 individuals in total) selected from each sampled population were carefully cleaned with micro needles of any impurities and soaked in distilled water for 10 minutes. DNA extraction was then performed using the BIORON GmbH "Ron's Tissue DNA Mini Kit" following the protocol provided by the manufacturer for all the specimens except for one female from Seixal (Madeira, Portugal) to be used as neotype of the species. Total DNA was extracted from this last specimen following the protocol described by Cornils (2015), slightly modified. In fact, in order to grant the long-term conservation of the exoskeleton of the neotype, upon its staining with chlorazol black, the specimen was moved in 96% ethanol through a "drop-by-drop" substitution of the implemented lysis buffer. The extracted DNA was amplified by polymerase chain reaction (PCR). A fragment of the mitochondrial marker *12S ribosomal RNA* gene *(12S)* and a fragment of the nuclear gene *28S ribosomal RNA* gene *(28S)* were chosen for amplification by PCR. The primer pair "L13337-12S" (5′-YCT ACT WTG YTA CGA CTT ATC TC-3′) and "H13845-12S" (5′-GTG CCA GCA GCT GCG GTT A-3′; Machida et al., 2002) was used to amplify a fragment of the *12S*. The primer set "28S-F1a" (5′-GCG GAG GAA AAG AAA CTA AC-3′) and "28S-R1a" (5′-GCA TAG TTT CAC CAT CTT TCG GG-3′) (Ortman, 2008) was used to amplify a fragment of the *28S*. The PCR for the *12S* was carried out with 30 cycles of a 25 µl reaction volume containing 18 µl of distilled water, 3 µl of 10X Buffer including 15 mM of $MgCl_2$, 0.5 µl of dNTPs (10 mM each), 0.5 µl of each of the primers (10 µM), 0.5 µl of Taq polymerase (5 U/µl) and 2 µl of DNA template, for a total volume of 25 µl. The thermal cycle consisted of an initial 5 min denaturation phase at 95 °C, followed by 30 cycles with a denaturation at 96 °C for 15 s, annealing at 45 °C for 30 s, and extension at 72 °C for 15 s, plus a final extension cycle of 5 min to 72 °C. The composition of the PCR mix for the *28S* included 18.75 µl of distilled water, 2.5 µl of Buffer 10X (including 15 mM of $MgCl_2$), 0.3 µl of dNTPs (10 mM each), 0.3 µl of each primer (10 µM), 0.35 µl of Taq polymerase (5 U/µl), and 2.5 µl of DNA template, for a total volume of 25 µl. The thermal cycle consisted of an initial denaturation phase at 95 °C, with a duration of 5 min. This is followed by 35 denaturation cycles (95 °C, 1 min), annealing (48 °C, 1 min), and extension (72 °C, 1 min), plus a final extension cycle of 8 min at 72 °C. Subsequently, 5 µl of each PCR product was used to perform electrophoresis on 2% agarose gel, with a voltage of 90 V, for 20 min. The outcome of the electrophoresis was verified using a UV transilluminator. The samples that showed a single clear band with the expected length for each marker used were purified using the Exo-SAP-IT® kit (Affymetrix USB). Sequencing was operated by Macrogen Inc. (Madrid, Spain) via an ABI 3130xL sequencer (Applied Biosystems). The same primers used for the PCR were used for the direct sequencing of the PCR products. The quality of the resulting chromatograms was verified by measuring their "Phred score" value (Richterich, 1998). Among these, only the sequences that showed continuous readings

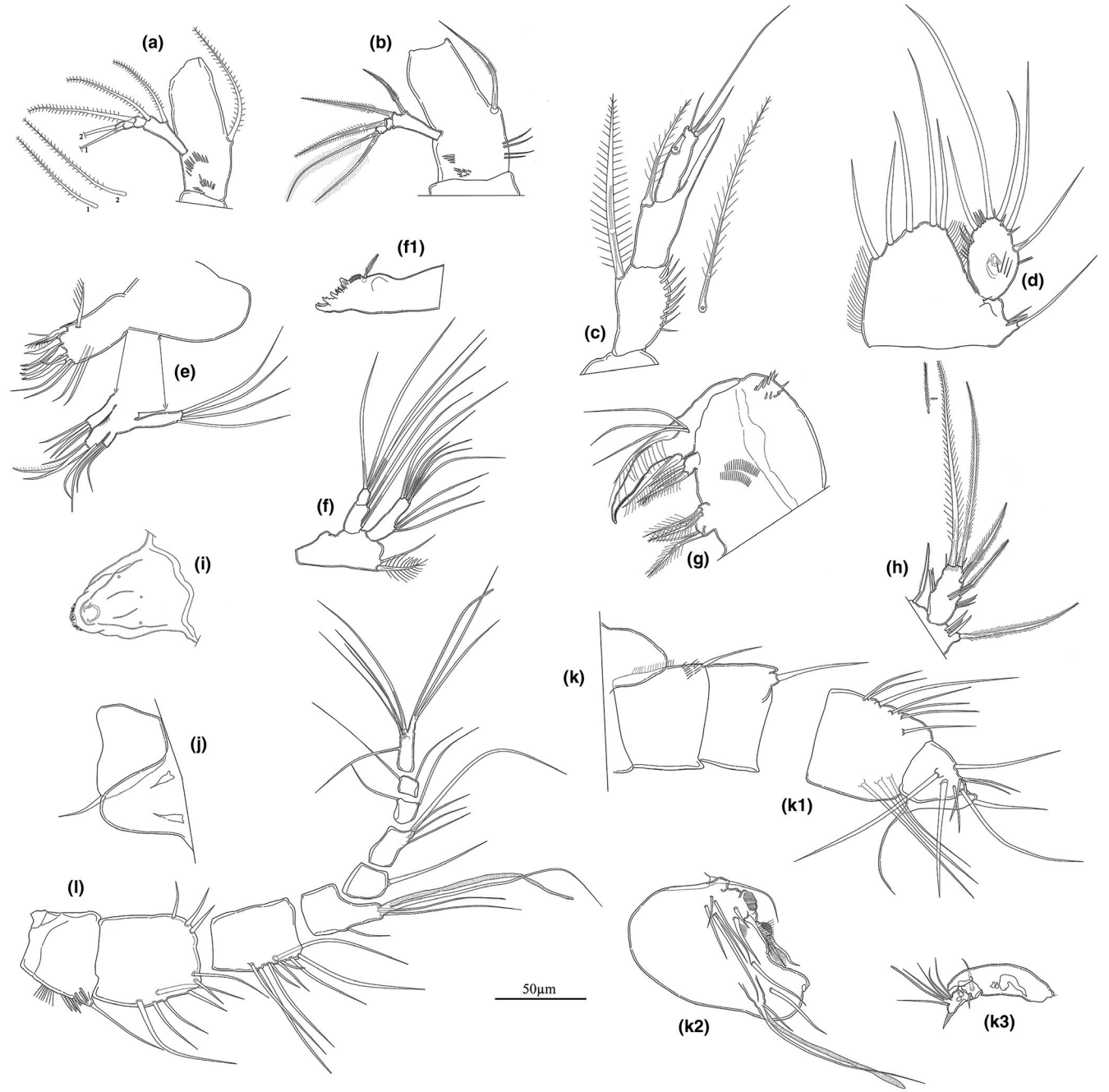

FIGURE 5 *Tigriopus brevicornis* (Sanxenxo, Spain—GLC): Female, antennal baseoendopodite and exopodite (a). *Tigriopus fulvus* s.s. (Seixal, Madeira, Portugal—SXL): Female, baseoendopodite and exopodite antenna (b), fifth pair of legs (P5) (d), maxillula (e), mandible (f), maxilla (g), labrum (i), rostrum (j), antennule (l). Male, second pair of legs (P2) (c), P5 endopodite (h), antennule (k)

of high-quality bases (QV > 20) were kept. The software Chromas v. 2.6.2 (Technelysium, Pty. Ltd. 1998, Queensland, Australia) was used for chromatogram analysis. Overall, 74 *12S* sequences of *Tigriopus fulvus*, six of *T. brevicornis*, and one of *T. californicus* were obtained. In addition, 34 *28S* sequences of *T. fulvus*, four of *T. brevicornis*, and one of *T. californicus* were produced. The only *Tigriopus* cf. *fulvus 28S* sequence available on GenBank (Accession Number, A.N., EU370444) was downloaded and included in the analyses. All sequences were aligned using the software MEGAX (Kumar et al., 2018) using the ClustalW method (Thompson et al., 1994). All the novel sequences were deposited in GenBank (see Table 2 for their A.N.). Alignments are available as Alignments S1–S3.

The incongruence length difference test (ILD, Farris et al., 1995) as implemented in PAUP* v. 4.0b10 (Swofford, 2002) was used to test whether the mitochondrial and nuclear fragments could be combined into one dataset. According to Cunningham (1997), if $p > 0.01$, pooling the data improves the phylogenetic accuracy and thus it is admissible to merge the tested datasets into a single matrix. With $p = 0.82$ this condition was fulfilled, and the *12S* and *28S* datasets were thus also analyzed jointly ("combined

TABLE 2 Origin and GenBank accession numbers for the analyzed *Tigriopus* specimens. Geographic coordinates are expressed as decimal degrees (Map Datum: WGS84)

Locality code	Sample code	Geographic origin	Accession Number		Source
			12S	*28S*	
Tigriopus fulvus					
TIP[a]	TF44	Algeria, Tipaza	MN625569	–	Present work
TIP[a]	TF45	Algeria, Tipaza	MN625570	MN606246	Present work
ROV[b]	TF54	Croatia, Rovinj	MN625576	MN606250	Present work
ROV[b]	TF53	Croatia, Rovinj	MN625575	–	Present work
ROV[b]	TF65	Croatia, Rovinj	MN625577	–	Present work
TER	TF1	Italy, Terrasini	MN625543	–	Present work
TER	TF6	Italy, Terrasini	MN625544	–	Present work
TER	TF7	Italy, Terrasini	MN625542	MN606226	Present work
LIN	TF104	Italy, Linosa	MN625550	MN606229	Present work
LIN	TF105	Italy, Linosa	MN625549	MN606230	Present work
GRA	TF97	Italy, Torretta Granitola	MN625603	MN606259	Present work
GRA	TF98	Italy, Torretta Granitola	MN625604	–	Present work
BAR	TF2	Italy, Barcarello	MN625553	–	Present work
BAR	TF8	Italy, Barcarello	MN625555	–	Present work
BAR	TF9	Italy, Barcarello	MN625554	MN606227	Present work
MAG	TF10	Italy, Magnisi	–	MN606232	Present work
MAG	TF11	Italy, Magnisi	MN625548	–	Present work
PLE	TF4	Italy, Plemmirio	MN625540	–	Present work
PLE	TF12	Italy, Plemmirio	MN625539	–	Present work
PLE	TF13	Italy, Plemmirio	MN625541	MN606238	Present work
MIL	TF14	Italy, Milazzo	MN625546	MN606239	Present work
MIL	TF15	Italy, Milazzo	MN625547	–	Present work
MIL	TF16	Italy, Milazzo	MN625545	–	Present work
COR	TF17	Italy, Cornino	MN625551	MN606240	Present work
COR	TF19	Italy, Cornino	MN625552	–	Present work
UST	TF23	Italy, Ustica	MN625556	MN606241	Present work
UST	TF24	Italy, Ustica	MN625557	–	Present work
PAN1	TF26	Italy, Pantelleria	MN625558	–	Present work
PAN2	TF27	Italy, Pantelleria	MN625559	MN606242	Present work
TRI	TF32	Italy, Tricase	MN625565	–	Present work
TRI	TF37	Italy, Tricase	MN625564	–	Present work
TRI	TF101	Italy, Tricase	MN625566	MN606228	Present work
CEF	TF38	Italy, Cefalù	MN625567	MN606244	Present work
CEF	TF39	Italy, Cefalù	MN625568	–	Present work
POR	TF43	Italy, Portoscuso	MN625605	MN606245	Present work
CAS	TF46	Italy, Castiglioncello	MN625532	MN606247	Present work
GAR	TF83	Italy, Gargano	MN625588	MN606253	Present work
GAR	TF84	Italy, Gargano	MN625589	MN606254	Present work
USA	TF92	Italy, Usai	MN625597	MN606258	Present work
USA	TF93	Italy, Usai	MN625598	–	Present work
GEN	TF94	Italy, Genova Nervi	MN625599	–	Present work
GEN	TF95	Italy, Genova Nervi	MN625600	–	Present work

(Continues)

TABLE 2 (Continued)

Locality code	Sample code	Geographic origin	Accession Number		Source
			12S	*28S*	
GEN	TF99	Italy, Genova Nervi	MN625601	–	Present work
GEN	TF102	Italy, Genova Nervi	MN625602	–	Present work
KOK	TF89	Greece, Kokkinoreia	MN625594	MN606257	Present work
KOK	TF90	Greece, Kokkinoreia	MN625595	–	Present work
KOK	TF91	Greece, Kokkinoreia	MN625596	–	Present work
SDI	TF85	Morocco, Sidi Ifni	MN625590	MN606255	Present work
SDI	TF86	Morocco, Sidi Ifni	MN625591	–	Present work
CPT	TF87	Morocco, Cape Tamri	MN625592	MN606256	Present work
CPT	TF88	Morocco, Cape Tamri	MN625593	–	Present work
SXL[c]	TF58	Portugal, Seixal (Farrobo)	MN625578	MN606251	Present work
SXL[c]	TF59	Portugal, Seixal (Farrobo)	MN625580	–	Present work
SXL[c]	TF72	Portugal, Seixal (Farrobo)	MN625579	–	Present work
PDC[c]	TF60	Portugal, Porto da Cruz	MN625581	–	Present work
PDC[c]	TF61	Portugal, Porto da Cruz	MN625583	–	Present work
PDC[c]	TF67	Portugal, Porto da Cruz	MN625584	MN606252	Present work
PDC[c]	TF73	Portugal, Porto da Cruz	MN625582	–	Present work
JAV	TF49	Spain, Jàvea	MN625571	MN606248	Present work
JAV	TF50	Spain, Jàvea	MN625572	–	Present work
BEN	TF51	Spain, Benitachell	MN625573	–	Present work
BEN	TF52	Spain, Benitachell	MN625574	MN606249	Present work
MEN	TF76	Spain, Menorca	MN625585	–	Present work
MEN	TF77	Spain, Menorca	MN625586	–	Present work
MEN	TF78	Spain, Menorca	MN625587	–	Present work
AKA	TF109	Cyprus, Ammos tou Kambouri	MN625533	MN606231	Present work
AKA	TF110	Cyprus, Ammos tou Kambouri	MN625534	MN606233	Present work
FKP	TF111	Cyprus, Faros—Kato Paphos	MN625535	MN606234	Present work
FKP	TF112	Cyprus, Faros—Kato Paphos	MN625536	MN606235	Present work
YEO	TF113	Cyprus, Agios Georgios	MN625537	MN606236	Present work
YEO	TF114	Cyprus, Agios Georgios	MN625538	MN606237	Present work
BIZ	TF28	Tunisia, Bizerte	MN625560	MN606243	Present work
BIZ	TF29	Tunisia, Bizerte	MN625561	–	Present work
BIZ	TF30	Tunisia, Bizerte	MN625562	–	Present work
BIZ	TF34	Tunisia, Bizerte	MN625563	–	Present work
T. brevicornis					
GLC	TF79	Spain, Sanxenxo	MN625528	MN606222	Present work
GLC	TF80	Spain, Sanxenxo	MN625529	–	Present work
GLC	TF81	Spain, Sanxenxo	MN625530	MN606223	Present work
GLC	TF82	Spain, Sanxenxo	MN625531	–	Present work
TRD	TF107	Spain, Sanxenxo	MN625526	MN606224	Present work
TRD	TF108	Norway, Trondheim	MN625527	MN606225	Present work
–	–	Norway, Trondheim	–	EU370444	Reumont et al., 2009
T. californicus					
TCL	TF106	Spain, Galicia	MN625525	MN606221	Present work

[a]Specimens from the type locality of *Tigriopus fulvus algiricus*

[b]Specimen from the type locality of *Tigriopus fulvus adriaticus*

[c]Specimens from Madeira, the *terra typica* of *Tigriopus fulvus* s.s.

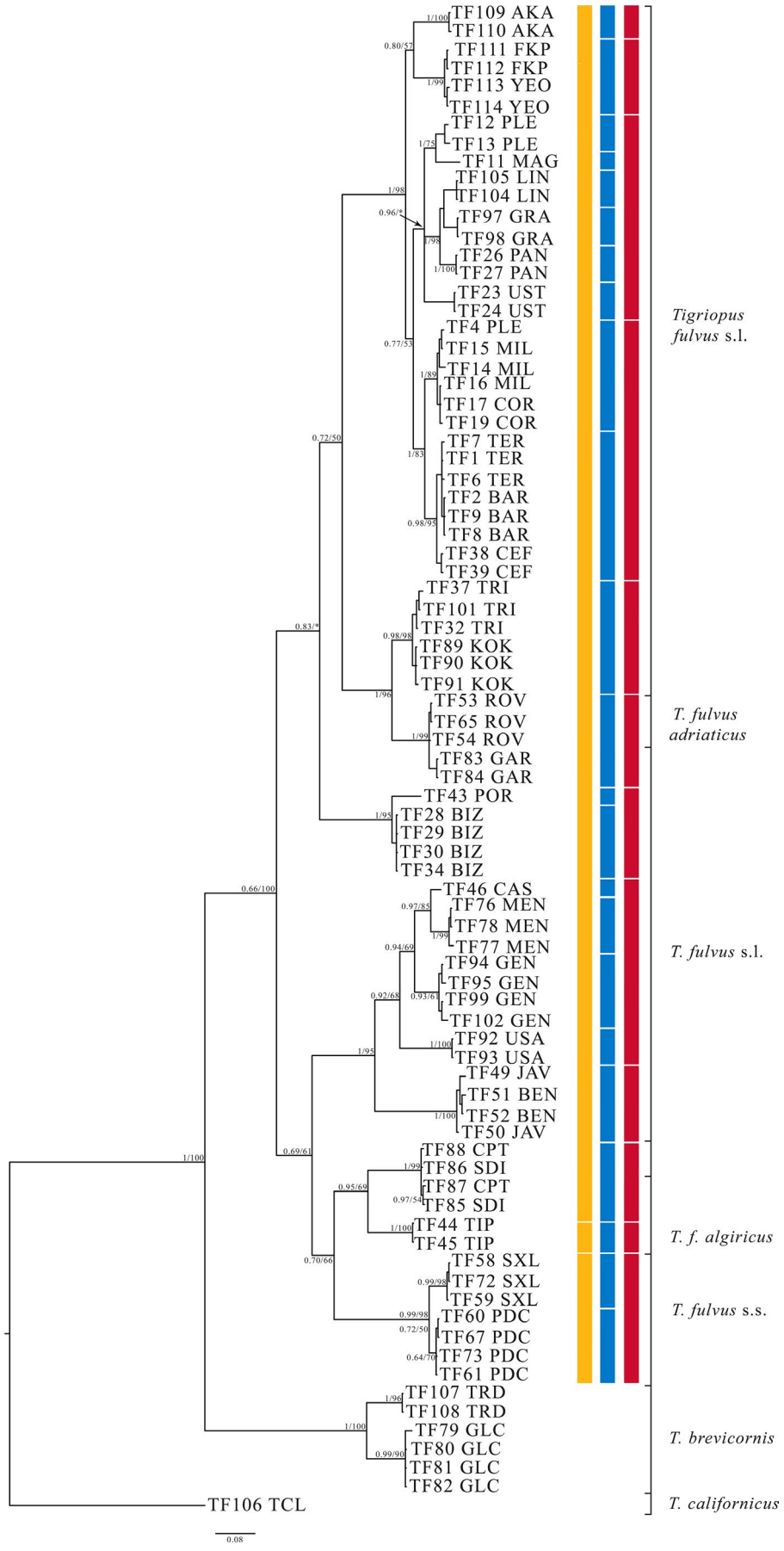
TF109 AKA
TF110 AKA
TF111 FKP
TF112 FKP
TF113 YEO
TF114 YEO
TF12 PLE
TF13 PLE
TF11 MAG
TF105 LIN
TF104 LIN
TF97 GRA
TF98 GRA
TF26 PAN
TF27 PAN
TF23 UST
TF24 UST
TF4 PLE
TF15 MIL
TF14 MIL
TF16 MIL
TF17 COR
TF19 COR
TF7 TER
TF1 TER
TF6 TER
TF2 BAR
TF9 BAR
TF8 BAR
TF38 CEF
TF39 CEF
TF37 TRI
TF101 TRI
TF32 TRI
TF89 KOK
TF90 KOK
TF91 KOK
TF53 ROV
TF65 ROV
TF54 ROV
TF83 GAR
TF84 GAR
TF43 POR
TF28 BIZ
TF29 BIZ
TF30 BIZ
TF34 BIZ
TF46 CAS
TF76 MEN
TF78 MEN
TF77 MEN
TF94 GEN
TF95 GEN
TF99 GEN
TF102 GEN
TF92 USA
TF93 USA
TF49 JAV
TF51 BEN
TF52 BEN
TF50 JAV
TF88 CPT
TF86 SDI
TF87 CPT
TF85 SDI
TF44 TIP
TF45 TIP
TF58 SXL
TF72 SXL
TF59 SXL
TF60 PDC
TF67 PDC
TF73 PDC
TF61 PDC
TF107 TRD
TF108 TRD
TF79 GLC
TF80 GLC
TF81 GLC
TF82 GLC
TF106 TCL
0.08
Tigriopus fulvus s.l.
T. fulvus adriaticus
T. fulvus s.l.
T. f. algiricus
T. fulvus s.s.
T. brevicornis
T. californicus

FIGURE 6 Bayesian phylogram (50% majority rule consensus tree) for *Tigriopus* spp. based on a 415-bp fragment of the mitochondrial *12S ribosomal RNA* gene. A sample of *Tigriopus californicus* was used as out-group to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian inference of phylogeny, BI)/bootstrap values (maximum likelihood, ML). Asterisks indicate bootstrap support values lower than 50. Rectangles refer to MOTUs as indicated by the K/Θ ratio (yellow rectangles), ABGD (blue rectangles), and mPTP (red rectangles). Square brackets group the samples according to the current taxonomy of the genus. Codes of the analyzed specimens refer to Table 2

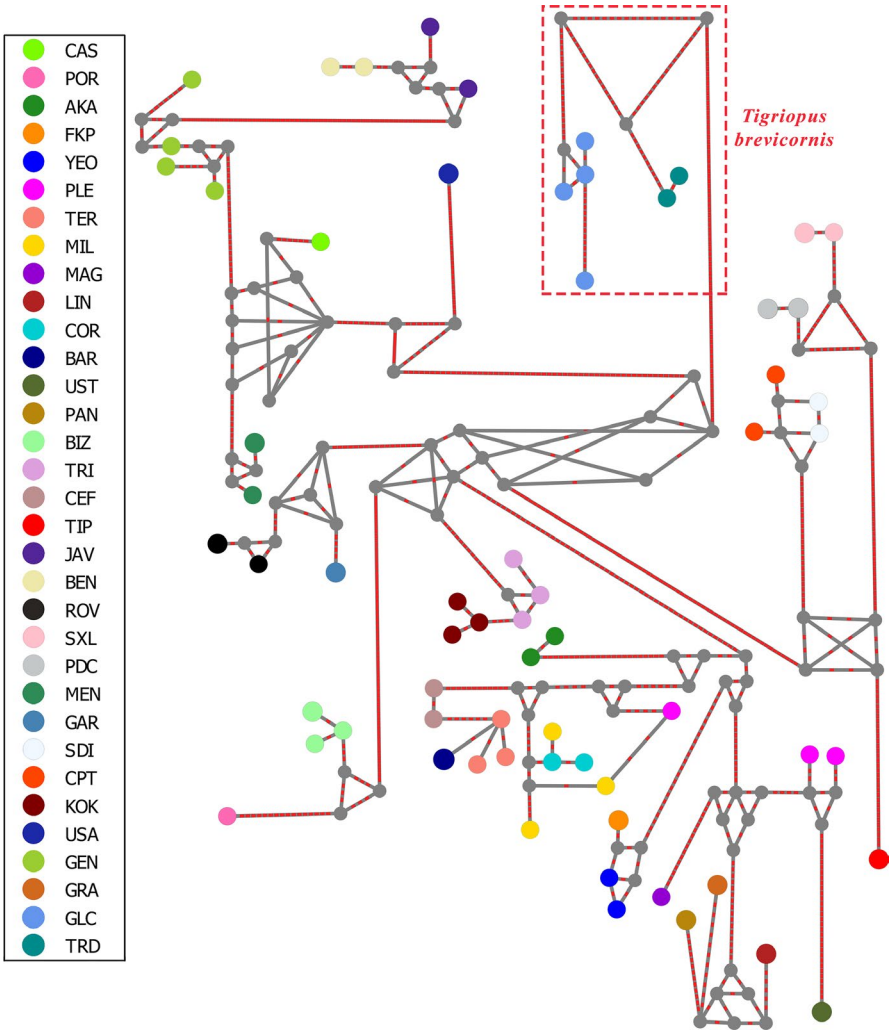

FIGURE 7 Median-joining haplotype network based on a 415-bp long fragment of the mitochondrial *12S ribosomal RNA* gene of *Tigriopus fulvus* and *T. brevicornis*. Substitution steps are shown in red. Each circle represents a haplotype. Codes of the analyzed populations refer to Table 1

dataset"). For the *12S* and *28S* datasets and the combined dataset, the software packages MrBayes v. 3.2.6 (Ronquist et al., 2012) and PhyML v. 3 (Guindon et al., 2010) were used for inferring phylogenetic relationships through Bayesian inference of phylogeny (BI) and maximum likelihood analysis (ML). As support measures for the nodes, bootstrap values (Felsenstein, 1985) were calculated with 1000 replicates in the ML trees, whereas in the BI tree, the posterior probability values were reported. PartitionFinder v. 1.0.1 (Lanfear et al., 2012) was used to choose the best evolutionary model following the "Akaike Information Criterion" (AIC; Akaike, 1974). For both the mitochondrial and nuclear fragments, and in the combined dataset, a general time-reversible model of sequence evolution with a proportion of invariable sites and gamma-distributed rate variation among sites was used (GTR+I+Γ; nst = 6) for both the BI and ML analyses. In the BI analyses, two independent Markov Chain Monte Carlo analyses were performed with 1 million of generations (temp.: 0.2; default priors). Trees and parameter values were sampled every 100 generations, with the result of 10,000 trees for each analysis. The convergence in the analysis was reached (effective sample size (ESS) greater than 200 in all the analyses performed). The initial 25% of trees were discarded as "burn-in."

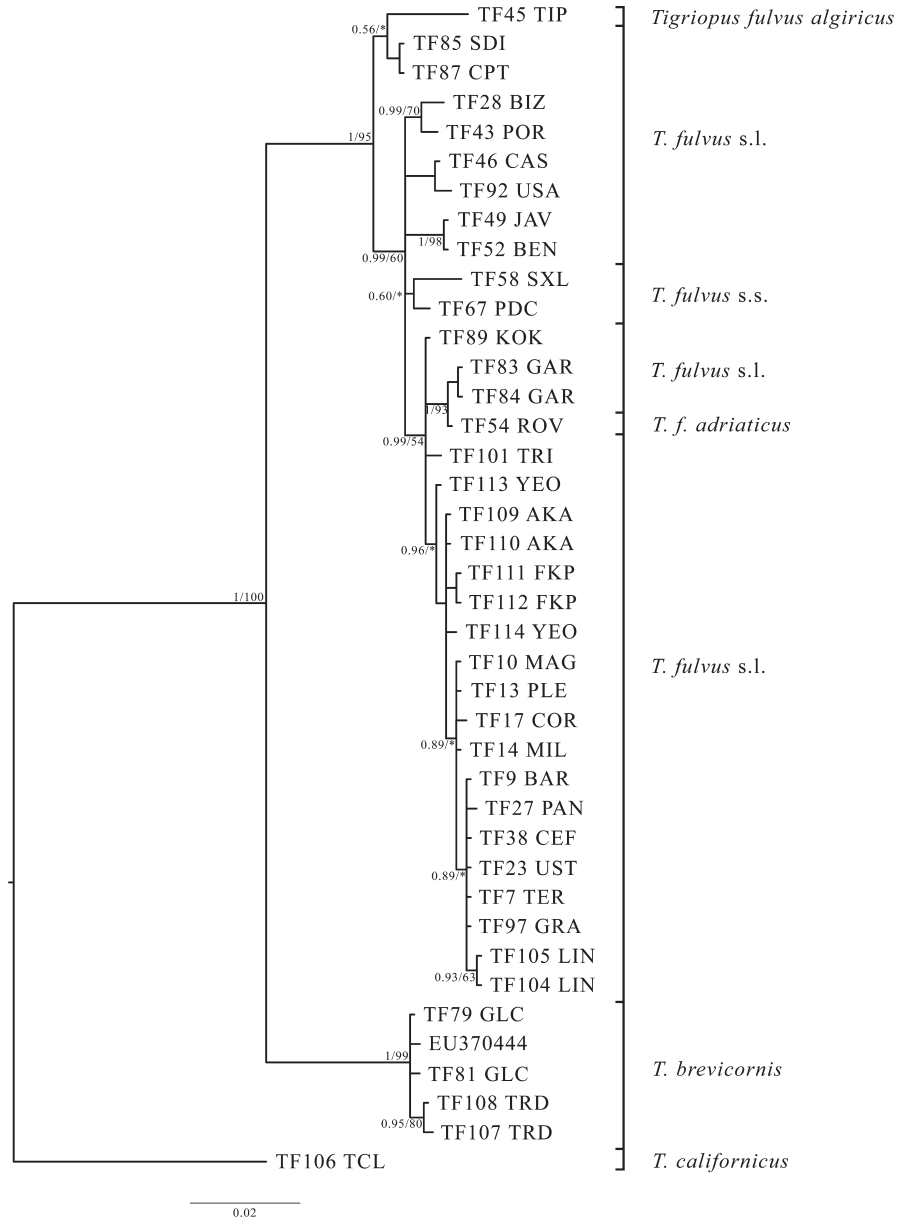

FIGURE 8 Bayesian phylogram (50% majority rule consensus tree) for *Tigriopus* spp. based on the 724-bp fragment of the nuclear *28S ribosomal RNA* gene. A sample of *Tigriopus californicus* was used as out-group to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian inference of phylogeny, BI)/bootstrap values (maximum likelihood, ML). Asterisks indicate bootstrap support values lower than 50. Square brackets group the samples according to the current taxonomy of the genus. Codes of the analyzed specimens refer to Table 2

In the frame of this paper, we followed the "evolutionary genetic species concept" proposed by Birky et al. (2010) in order to investigate the taxonomy and diversity of the studied populations. According to this concept, species are inclusive populations that are evolving independently from each other, either because they are reproductively isolated, or because they are separated by environmental or physical barriers, or both. Those lineages that evolve separately from others are thus considered different taxa of putative species rank.

Following Belaiba et al. (2019 and reference therein), the identification of the "Molecular Operational Taxonomic Units (MOTUs)," that is, putative species, was carried out implementing DNA taxonomy approaches based on different assumptions: a quantitative approach based on a distance-based model ("ABGD"; Puillandre et al., 2012); a phylogenetic criterion based on branching rates ("mPTP"; Kapli et al., 2017); and a population genetic criterion based on genetic isolation ("K/Θ ratio"; Birky, 2013; Birky et al., 2010). The three aforementioned taxonomic approaches were used for *12S* sequences only. ABGD and mPTP were performed through their online interfaces (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html and https://mptp.h-its.org/#/tree). Following Korn and Hundsdoerfer (2016), the K/Θ ratio was computed based on the uncorrected "*p*" distance matrix both within and among the detected clades. This method tests if the reciprocal monophyly of sister lineages is statistically significant, which would suggest that

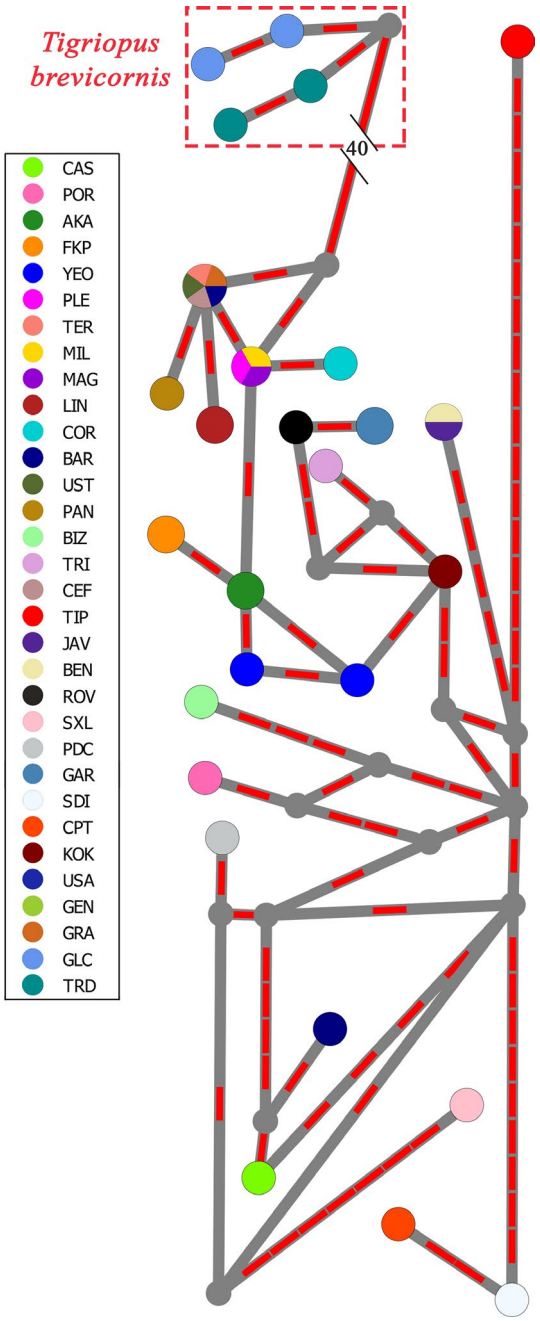

FIGURE 9 Median-joining haplotype network based on a 724-bp long fragment of the nuclear *28S ribosomal RNA* gene of *Tigriopus fulvus* and *T. brevicornis*. Substitution steps are shown in red. Each circle represents a haplotype. Codes of the analyzed populations refer to Table 1

they are independently evolving entities, hence *bonae species sensu* Birky et al. (2010).

It must be taken into account that these implemented DNA taxonomy approaches can be influenced by the number of individuals included in the analyses and by their geographic distribution, thus with the risk of over- or underestimating the actual number of taxa that occur in the studied dataset (Kapli et al., 2017; Puillandre et al., 2012; Zhang et al., 2013). Despite this *caveat*, we decided to use these methods in order to obtain a tentative picture of the distribution of the genetic diversity of *Tigriopus fulvus*.

Finally, for mitochondrial and nuclear sequences, haplotype networks were created with HaplowebMaker (https://eeg-ebe.github.io/HaplowebMaker/) with the "Median-Joining" method (Spöri & Flot, 2020).

3 | RESULTS

3.1 | Morphological identification

The sampling activities led to the collection of *Tigriopus fulvus* in 31 sites and *T. brevicornis* in two sites (Table 1 and Figure 1). The validity and constancy of the diagnostic morphological characters which allow to distinguish *T. fulvus* and *T. brevicornis* according to Carli and Fiori (1977) were confirmed in all the studied populations.

Based on our observations, the most evident characters to distinguish *T. brevicornis* from *T. fulvus* are as follows: (i) spinules are absent on the surface of the female exopodite of the fifth pair of legs (P5) in *T. brevicornis*, whereas they are present in *T. fulvus* (Figure 2); (ii) P5 setae are longer in *T. brevicornis* than in *T. fulvus* (see Figure 2d); and (iii) four spinules are present on the female baseoendopodite of the antenna of *T. fulvus*, which are absent in *T. brevicornis* (Figure 5a and b).

In all *T. fulvus* populations, a noteworthy intraspecific variability was found for those morphological characters considered diagnostic of the subspecies of *T. fulvus* (see Van Douwe, 1913 and Monard, 1935), for example, the width of the baseoendopodal lobe of female P5 (Figures 2-4).

The *T. californicus* samples from a commercial strain included in present analysis showed the characteristic morphology of the species.

3.2 | Molecular genetic analyses

The length of the *12S* PCR product ranged from 410 to 440 bp, and the length of the *28S* PCR product ranged from 870 to 890 bp. After having trimmed out the tails of the sequences, a properly aligned *12S* alignment (415 sites; Alignment S1) and a *28S* alignment (724 sites; Alignment S2) were obtained.

The *12S* phylogenetic trees obtained based on BI and ML analyses were rooted on *Tigriopus californicus* (Figure 6). All the *T. fulvus* s.l. specimens are grouped in a single clade, and the specimens from *T. fulvus algiricus* and *T. fulvus adriaticus loci typici* are nested within *T. fulvus* s.l. clade. In addition, it is possible to observe a strong genetic structuring, with pairwise uncorrected *"p"* interclade distance values ranging from 0 to 22%. The *12S* haplotype network shows private haplotypes for each rock pool with a maximum number of 68 evolutionary steps between haplotypes of the ingroup (Figure 7).

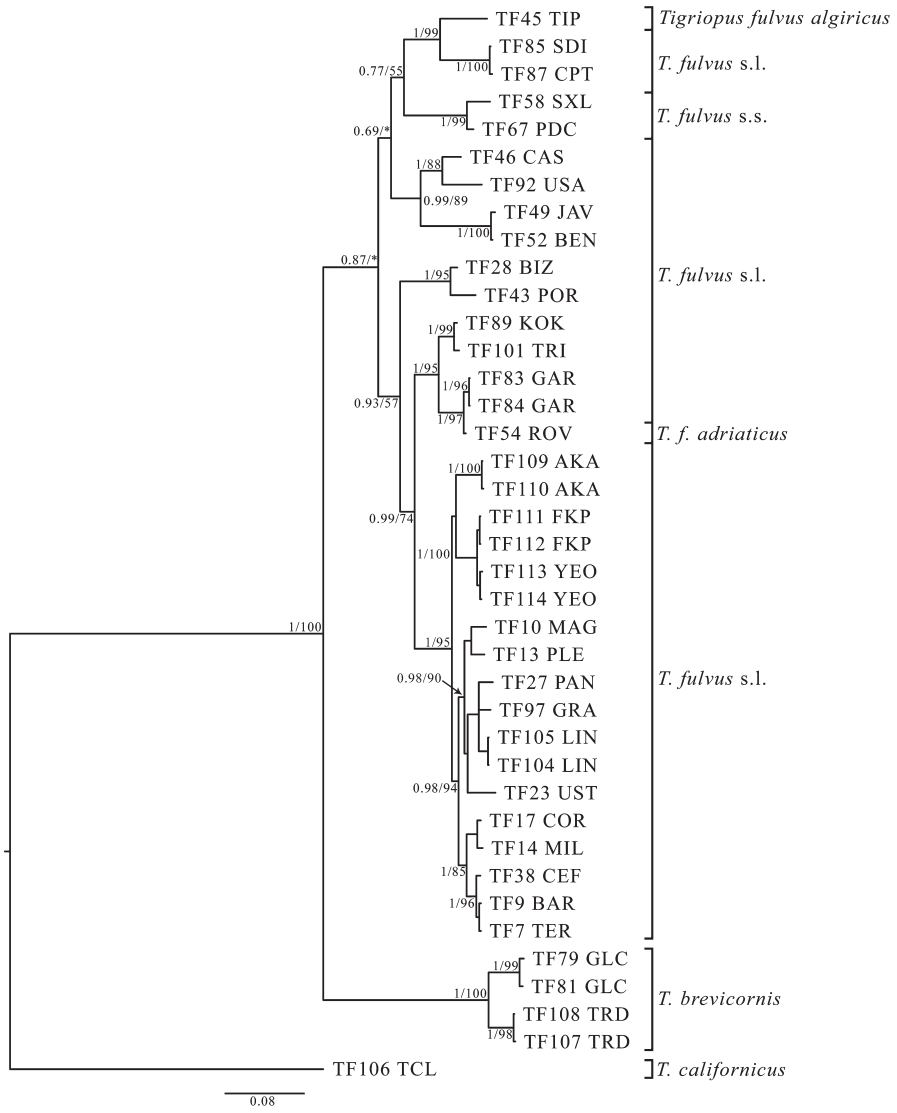

FIGURE 10 Bayesian phylogram (50% majority rule consensus tree) for *Tigriopus* spp. based on the 1139-bp fragment of the combined mito-nuclear DNA dataset. A sample of *Tigriopus californicus* was used as out-group to root the tree. Node statistical support is reported as nodal posterior probabilities (Bayesian inference of phylogeny, BI)/bootstrap values (maximum likelihood, ML). Asterisks indicate bootstrap support values lower than 50. Square brackets group the samples according to the current taxonomy of the genus. Codes of the analyzed specimens refer to Table 2

The *28S* phylogenetic trees based on BI/ML analyses were rooted on *Tigriopus californicus* (Figure 8). A clear separation between the clade that includes specimens of *T. brevicornis* and that of *T. fulvus* s.l. can be observed. It is noteworthy to note that the specimen from Galicia referred to as *T.* cf. *fulvus* by Reumont et al. (2009) (GenBank A.N. EU370444) is actually included into the clade of our *T. brevicornis* samples in good accordance to its geographic origin. The two alleged subspecies of *T. fulvus* are nested within the *T. fulvus* s.l. clade.

The haplotype network for the *28S* nuclear marker (Figure 9) shows phylogenetic relationships among populations similar to those shown by the BI/ML trees, with a maximum number of 17 evolutionary steps within the ingroup. In spite of the conservative nature of this marker, there are only three shared haplotypes among the populations.

The BI and ML trees based on the combined mito-nuclear DNA dataset and rooted on *T. californicus* showed a concordant topology with those obtained based on the single-marker analyses, with a monophyletic *Tigriopus fulvus* clade characterized by a high genetic structuring (Figure 10).

3.3 | DNA Taxonomy

The ABGD analysis suggests the existence of 23 species-level entities within the ingroup, with a value of "*P*" (prior maximal divergence of intraspecific diversity values) of 0.0139 (Figure S1). Conversely, the multi-rate Poisson Tree Process (mPTP) analysis reports the existence of 12 species-level entities (Figure S2) within the ingroup.

TABLE 3 Application of the "K/Θ ratio" to *Tigriopus fulvus* s.l. mitochondrial *12S* fragment

Group	n	p-dist	π	4\3π	Θ	K	K\Θ ratio	Sample
Tfulvcm	25	0.071	0.074	0.098	0.082	0.101	1.230	TF1; TF2; TF4; TF6; TF7; TF8; TF9; TF11; TF12; TF13; TF14; TF15; TF16; TF17; TF19; TF23; TF24; TF26; TF27; TF38; TF39; TF97; TF98; TF105; TF104
Tfadr	11	0.112	0.168	0.224	0.686	0.199	2.431	TF32; TF37; TF53; TF54; TF65; TF83; TF84; TF89; TF90; TF91; TF101
Tfulvsp1	6	0.057	0.069	0.092	0.076	0.189	2.482	TF109; TF110; TF111; TF112; TF113; TF114
Tfulvsp2	42	0.121	0.124	0.165	0.148	0.217	1.463	Tfulvcm + Tfadr + Tfulvsp1
Tfulvsp3	5	0.023	0.029	0.039	0.030	0.217	1.463	TF28; TF29; TF30; TF34; TF43
Tfulvsp4	47	0.144	0.147	0.196	0.183	0.290	1.585	Tfulvsp2 + Tfulvsp3
Tfulvsp5	14	0.127	0.137	0.183	0.168	0.290	1.585	TF49; TF46; TF50; TF51; TF52; TF76; TF77; TF78; TF92; TF93; TF94; TF95; TF99; TF102
Tfulvsp6	4	0.006	0.008	0.010	0.008	0.305	1.665	TF85; TF86; TF87; TF88
Tfalg[a]	2	0.002	0.004	0.006	0.004	0.159	19.402	TF44; TF45
Tfulvss	7	0.025	0.029	0.038	0.030	0.251	8.341	TF58; TF59; TF60; TF61; TF67; TF72; TF73
Tfulvsl1	47	0.140	0.143	0.190	0.176	0.293	0.943	Tfulvsp4
Tfulvusl2	27	0.212	0.220	0.293	0.311	0.293	0.943	Tfulvsp5 + Tfulvsp6 + Tfalg + Tfulvss

Abbreviations: n: number of individuals; p-dist: uncorrected p-distance; π: nucleotide diversity; Θ: intra-clade variation; K: interclade distances; Tfulvcm: *T. fulvus* from the central Mediterranean area; Tfadr: *T. f. adriaticus*; Tfalg: *T. f. algiricus*; Tfulvss: *T. fulvus* s.s. from Madeira; Tfulvsl: *T. fulvus* s.l.
[a]p-dist corrected using 1/L, where "L" is the length of the fragment (cf. Birky, 2013).

In sharp contrast with the mPTP and ABGD results, the K/Θ ratio reported values lower than "4" in the inter-groups relations, thus suggesting the existence of a single, albeit genetically heavily structured, species (Table 3).

4 | DISCUSSION

4.1 | Taxonomical remarks

Based on the morphological comparison between the specimens originating from the topotypical populations of the two *T. fulvus* subspecies with those from Madeira, the *terra typica* of *T. fulvus* s.s., no morphological differences emerged. The individuals of the population of Madeira, as well as all sampled *Tigriopus fulvus* s.l. from the Atlantic–Mediterranean sites, share the morphological characters that would hypothetically define *T. fulvus adriaticus* and *T. fulvus algiricus*. In fact, *T. fulvus adriaticus* should be characterized by a baseoendopodite of female P5 wider than long and with roundish exopodite, by female caudal rami ornamented with spinules, and by the presence of a row of spinules on the outer surface of the male P5 exopodite (Van Douwe, 1913), whereas *T. fulvus algiricus* should be characterized by the baseoendopodal lobe of female P5 "*remarquablement plus large et moins avancé*" (noticeably larger and less advanced), not reaching the distal segment of the exopodite and by the second segment of the male endopodite of the second pair of legs (P2) always exceeding the distal apex of the third segment of the P2 exopodite (Monard, 1935). All these characters can be observed in topotypical specimens of *T. fulvus* (Figures 2-5), thus proving the lack of taxonomical relevance of the morphological characters used to allegedly characterize the subspecies *T. fulvus algiricus* and *T. fulvus adriaticus* (Monard, 1935; Van Douwe, 1913). In fact, these two subspecies were erroneously characterized using as comparative material specimens and drawings of *T. brevicornis* instead of *T. fulvus* (see above, and the comments provided by Božić, 1960, and Carli & Fiori, 1977).

The ABGD and mPTP DNA taxonomy approaches suggested the presence of an unlikely high number of taxa of putative species rank within the ingroup. Conversely, the K/Θ ratio based on the same gene suggests the existence of only a single, albeit highly structured, species. The different approaches of DNA taxonomy taken individually are not, however, sufficient to establish the rank to be attributed to the various lineages, since they are only "Primary Species Hypotheses" to be taken into account and tested. Therefore, it is preferable to use a combination of different, independent approaches and search for a consensus of results (Fontaneto et al., 2015; Marrone et al., 2020). In the absence of a consensus among the results of the different DNA taxonomy approaches used in the present work, it was chosen to follow the more conservative one, namely the K/Θ ratio (Birky & Barraclough, 2009; Bode et al., 2010; Vecchioni et al., 2019), whose accuracy, moreover, should not be significantly affected by the sample size (Birky, 2013). Accordingly, considering the absence of morphological differences among the alleged *Tigriopus fulvus* subspecies, and in the light of the outcomes of both phylogenetic analyses and DNA taxonomy approaches, the subspecies *T. fulvus adriaticus* Van Douwe, 1913 and *T. fulvus algiricus* Monard, 1935 should not be considered valid taxa; therefore, they are here considered junior synonyms of *T. fulvus* s.s..

To date, *T. lilljeborgii* Norman, 1869 is one of the synonyms attributed to *Tigriopus fulvus* (see query for *Tigriopus fulvus* in World Register of Marine species, WORMS, http://www.marinespecies.org/). However, this synonymy is likely erroneous, as the binomen *T. lilljeborgii* was attributed to English *Tigriopus* populations, most likely belonging to *T. brevicornis*. In addition, Mistakidis (1949) described the variety "*northumbriensis*" of *T. lilljeborgii*, that is not accepted since it was based on Sars's drawings (Carli & Fiori, 1977). Therefore, pending a dedicated study, the epithet "*lilljeborgii*" should be conservatively dissociated from *T. fulvus*, avoiding erroneous association that might prevent the understanding of the actual distribution patterns of *Tigriopus* species (e.g., see query for *Tigriopus fulvus* in WORMS, http://www.marinespecies.org/).

Finally, the *28S* sequence of the specimen identified as *Tigriopus* cf. *fulvus* by Reumont et al. (2009) actually clustered within our *T. brevicornis* clade (Figure 8), as also expected based on its geographic origin (Galicia, Spain), and it is thus to be ascribed to this last species.

4.2 | Designation of a neotype and of a restricted *locus typicus* for *Tigriopus fulvus*

Fischer (1860) did not establish a holotype for *T. fulvus* nor a precise *locus typicus* for the species. Our efforts to trace Fischer's collections were unsuccessful, so that the designation of a neotype and of a restricted *locus typicus* for the species can be proposed (Article 75.3 of the ICZN Code). The historical locality proposed by Fischer (1860) in the original description of the species is Madeira (Portugal), without indication of a precise location. Accordingly, a neotype from a rock pool in the municipality of Seixal (Madeira, Portugal) is here designated. This way the criteria of Article number 75.3 of the ICZN Code are satisfied and the neotype of *T. fulvus* is hereby formally established.

Description: The genus *Tigriopus* Norman, 1879 includes the species *T. fulvus* originally described by Fischer (1860) as *Harpacticus fulvus*. In accordance with the morphological characteristics already known (cf. Božić, 1960; Carli & Fiori, 1977; Fischer, 1860), the neotype and the paratypes, described in the present work, are easily recognizable (both male and female) by the presence of: i) four segments in the antennal exopod; ii) seven setae and spines on the distal segment of the exopodites of the second (P2) and third (P3) pair of legs, and eight setae and spines on the distal segment of the exopodite of the fourth (P4) pair of legs; and iii) four setae on the distal segment of the P3 and P4 endopodites. Furthermore, the fifth pair of legs (P5) has rounded exopodites, ornamented with two spinules row and short setae.

Genetics: The neotype is genetically determined by sequences of fragments of the mitochondrial *12S rRNA* gene and the nuclear *28S rRNA* gene (GenBank Accession Numbers: MN625578 and MN606251).

Restricted locus typicus: A supratidal rock pool in Farrobo, in the municipality of Seixal, northern coast of Madeira (Figure S3). Geographic WGS84 coordinates: 32°49'37.2"N 17°06'52.0"W.

Ecology and distribution: The species lives in supratidal and upper intertidal rock pools. Its present distribution, according to currently available data, covers all the Mediterranean Sea, and the Eastern Atlantic Ocean roughly between 33° and 29° latitude N.

Collection: The neotype is an adult female deposited in the Crustacean collection of the Zoology section "La Specola," Natural History Museum, University of Florence (MZUF 647). An allotype (MZUF 648) and ten paratypes (five female and five males, MZUF 649–650) were deposited in the same collection.

An updated taxonomical synopsis and list of synonyms is reported below:

Systematics
Family: Harpacticidae Dana, 1846
Genus: *Tigriopus* Norman, 1869
Type species of the genus: *Tigriopus brevicornis* (Müller, 1776) (sub *T. lilljeborgii* Norman, 1869)

Tigriopus fulvus (Fischer, 1860)
***Locus typicus*:** Seixal, Madeira (Portugal, Atlantic Ocean), Recent.
Synonyms
Harpacticus fulvus Fischer, 1860
Tigriopus fulvus var. *adriatica* van Douwe, 1913
Tigriopus fulvus var. *algirica* Monard, 1936

4.3 | Genetic diversity pattern

The phylogenetic analysis of the studied Atlantic–Mediterranean *Tigriopus fulvus* populations revealed a noteworthy geographic structuring of the genetic diversity for both implemented genetic markers. In addition, based on the *12S* dataset, pairwise uncorrected "*p*" distance value ranging between 0 and 22% was found, similarly to the ranges observed for *T. fulvus* mtDNA *COI (cytochrome c oxidase I)* sequences (see Vecchioni et al., 2019) and for other *Tigriopus* spp. (Edmands, 2001; Handschumacher et al., 2010; Ki et al., 2009). The high genetic diversity found within the species of the genus *Tigriopus* is also typical of other harpacticoid families (e.g., Parastenocarididae, Bruno et al., 2020 and reference therein).

Within *Tigriopus fulvus* s.l., a longitudinal pattern of the distribution of genetic diversity is evident, with the Atlantic and the westernmost Mediterranean populations being well characterized versus the Central and Eastern Mediterranean clades. However, it is likely that an increased sampling effort will lead to a more gradual longitudinal cline of the genetic diversity.

The strong geographically based structuring of the genetic diversity of the *Tigriopus* populations inhabiting the Atlantic–Mediterranean area, which is detectable even at very small geographic scale, is possibly compatible with the long-term persistence of

Tigriopus metapopulations inhabiting isolate rocky outcrops (Burton, 1997), and the realization of dedicated studies aimed at studying the extinction and recolonization patterns of *Tigriopus* populations inhabiting individual pools is highly desirable. Moreover, Fourdrilis and Backeljau (2019) recently proposed that the astonishingly high genetic diversity observed in another supratidal invertebrate, the gastropod *Melarhaphe neritoides* (Linnaeus, 1758), could be ascribed to the occurrence of very high mutation rates, that may conceal the signal of the gene flow possibly occurring; this phenomenon is called "hyperdiversity" by Fourdrilis and Backeljau (2019). Considering our datasets, we cannot rule out that a hyperdiversity phenomenon is actually in place for *Tigriopus fulvus*, possibly acting synergically with other processes. However, in the absence of exhaustive data it is currently impossible to corroborate this hypothesis. The investigation of the processes determining the diversity pattern observed in *Tigriopus* spp. is a promising research field, which should desirably be the object of dedicated, large-scale studies.

ACKNOWLEDGMENTS

We wish to thank Fabio Stoch (Université Libre de Bruxelles, Belgium) for the help he provided us with the collection of some samples of *Tigriopus fulvus* and for the stimulating discussions we had on the topic of this manuscript. Giuseppe Alfonso (University of Salento, Italy), Andrés Millán and Susana Pallarès (University of Murcia, Spain), Athina Papatheodoulou (I.A.CO Water & Environmental Consultants Ltd), Daniel Abed-Navandi (University of Vienna, Austria), Davide Spatafora, Eya Belaiba, and Seif Bououd kindly provided some of the studied samples. A first draft of present paper was significantly improved by the constructive criticisms of three anonymous reviewers.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the Supporting Information section of this article.

ORCID

Luca Vecchioni https://orcid.org/0000-0003-4325-9728
Marco Arculeo https://orcid.org/0000-0001-8911-6736
Vezio Cottarelli https://orcid.org/0000-0001-9925-0250
Federico Marrone https://orcid.org/0000-0002-4730-0452

REFERENCES

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, *19*(6), 716–723. https://doi.org/10.1109/TAC.1974.1100705.

Antonini, G., Audisio, P., Mancini, E., De Biase, A., Tronci, C., Rossetti, G., & Trizzino, M. (2010). Molecular phylogeography of two Italian sibling species of *Calobius* (Coleoptera, Hydraenidae, Ochthebiinae) inhabiting Mediterranean marine rock-pools. *Marine Biology*, *157*(2), 371–381. https://doi.org/10.1007/s00227-009-1324-9.

Audisio, P., Trizzino, M., De Biase, A., Rossetti, G., Mancini, E., & Antonini, G. (2010). Molecular and morphological evidence of a new sibling species of *Calobius* (Coleoptera: Hydraenidae) of the *C. quadricollis* complex from peninsular Italy. *Italian Journal of Zoology*, *77*(1), 29–37. https://doi.org/10.1080/11250000902845738.

Belaiba, E., Marrone, F., Vecchioni, L., Bahri-Sfar, L., & Arculeo, M. (2019). An exhaustive phylogeny of the combtooth blenny genus *Salaria* (Pisces, Blenniidae) shows introgressive hybridization and lack of reciprocal mtDNA monophyly between the marine species *Salaria basilisca* and *Salaria pavo*. *Molecular Phylogenetics and Evolution*, *135*, 210–221. https://doi.org/10.1016/j.ympev.2019.02.026.

Biandolino, F., Parlapiano, I., Faraponova, O., & Prato, E. (2018). Effects of short- and long-term exposures to copper on lethal and reproductive endpoints of the harpacticoid copepod *Tigriopus fulvus*. *Ecotoxicology Environment Safety*, *147*(2018), 327–333. https://doi.org/10.1016/j.ecoenv.2017.08.041.

Birky, C. W. Jr (2013). Species detection and identification in sexual organisms using population genetic theory and DNA sequences. *PLoS One*, *8*(1), e52544. https://doi.org/10.1371/journal.pone.0052544.

Birky, C. W. Jr, Adams, J., Gemmel, M., & Perry, J. (2010). Using population genetic theory and DNA sequences for species detection and identification in asexual organisms. *PLoS One*, *5*(5), e10609. https://doi.org/10.1371/journal.pone.0010609.

Birky, C. W. Jr, & Barraclough, T. G. (2009). Asexual Speciation. In I. Schön, K. Martens, & P. Dijk (Eds.), *Lost Sex*. Springer. https://doi.org/10.1007/978-90-481-2770-2_10.

Bode, S. N. S., Adolfsson, S., Lamatsch, D. K., Martins, M. J. F., Schmit, O., Vandekerkhove, J., Mezquita, F., Namiotko, T., Rossetti, G., Schön, I., Butlin, Rk., & Martens, K. (2010). Exceptional cryptic diversity and multiple origins of parthenogenesis in a freshwater ostracod. *Molecular Phylogenetic Evolution*, *54*(2), 542–552. https://doi.org/10.1016/j.ympev.2009.08.022.

Boxshall, G., & Defaye, D. (2020). *Fauna Europaea: Harpacticoida*, Tigriopus, Tigriopus fulvus. Fauna Europaea version 2020.06, https://fauna-eu.org.

Božić, B. (1960). *Le Genre* Tigriopus *Norman (Copépodes Harpacticoïdes) et ses forms européennes: recherches morphologiques et expérimentales*. Centre National de la Recherche Scientifique.

Brady, G. S. (1872). Contribution to the study of Entomostraca, VII. A list of non-parasitic Copepoda of the northeast coast of England. *Natural History Transaction Northumbria*, *4*(55), 432–445. https://doi.org/10.1080/00222937208696629.

Bruno, M. C., Cottarelli, V., Marrone, F., Spena, M. T., Vecchioni, L., & Grasso, R. (2020). Morphological and molecular characterization of three new Parastenocarididae (Copepoda: Harpacticoida) from caves in Southern Italy. *European Journal of Taxonomy*, *689*, 1–46. https://doi.org/10.5852/ejt.2020.689.

Burton, R. S. (1997). Genetic evidence for long term persistence of marine invertebrate populations in an ephemeral environment. *Evolution*, *51*(3), 993–998. https://doi.org/10.1111/j.1558-5646.1997.tb03681.x.

Carli, A., & Fiori, A. (1977). Morphological analysis of the two *Tigriopus* species found along the European coasts (Copepoda Harpacticoida). *Natura*, *68*(1–2), 101–110.

Chullasorn, S., Dahms, H. U., & Klangsin, P. (2013). A new species of *Tigriopus* (Copepoda: Harpacticoida: Harpacticidae) from Thailand with a key to the species of the genus. *Journal of Natural History*, *47*(5–12), 427–447. https://doi.org/10.1080/00222933.2012.757660.

Chullasorn, S., Ivanenko, V. N., Dahms, H. U., Kangtia, P., & Yang, W. X. (2011). A new species of *Tigriopus* (Copepoda, Harpacticoida, Harpacticidae) from Thailand with the description of its naupliar development. *Helgoland Marine Research*, *66*(2), 139–151. https://doi.org/10.1007/s10152-011-0254-3.

Cornils, A. (2015). Non-destructive DNA extraction for small pelagic copepods to perform integrative taxonomy. *Journal of Plankton Research*, *37*(1), 6–10. https://doi.org/10.1093/plankt/fbu105.

Cunningham, C. W. (1997). Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution*, *14*(7), 733–740. https://doi.org/10.1093/oxfordjournals.molbev.a025813.

Davenport, J., Barnett, P. R. O., & McAllen, R. J. (1997). Environmental tolerances of three species of the harpacticoid copepod genus *Tigriopus*. *Journal of the Marine Biological Association of the United Kingdom*, *77*(1), 3–16. https://doi.org/10.1017/S0025315400033749.

Dussart, B., & Defaye, D. (1990). Répertoire mondial des crustacés copépodes des eaux intérieures 3. *Harpacticoïdes*. *Crustaceana Supplement*, *16*, 1–384.

Edmands, S. (2001). Phylogeography of the intertidal copepod *Tigriopus californicus* reveals substantially reduced population differentiation at northern latitudes. *Molecular Ecology*, *10*(7), 1743–1750. https://doi.org/10.1046/j.0962-1083.2001.01306.x.

Farris, J. S., Kallersjo, M., Kluge, A. G., & Bult, C. (1995). Testing significance of incongruence. *Cladistics*, *10*(3), 315–319. https://doi.org/10.1111/j.1096-0031.1994.tb00181.x.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, *39*(4), 783–791. https://doi.org/10.2307/2408678.

Fischer, S. (1860). Beiträge zur Kenntnis der Entomostraceen. *Abhandlungen der Bayerischen Akademie der Wissenschaften*, *8*, 1–645.

Fontaneto, D., Flot, J. F., & Tang, C. Q. (2015). Guidelines for DNA taxonomy, with a focus on the meiofauna. *Marine Biodiversity*, *45*(3), 433–451. https://doi.org/10.1007/s12526-015-0319-7.

Fourdrilis, S., & Backeljau, T. (2019). Highly polymorphic mitochondrial DNA and deceiving haplotypic differentiation: implications for assessing population genetic differentiation and connectivity. *BMC Evolutionary Biology*, *19*, 92. https://doi.org/10.1186/s12862-019-1414-3.

Ganning, B., & Wulff, F. (1970). Measurements of community metabolism in some Baltic brackish water rockpools by means of diel oxygen curves. *Oikos*, *21*(2), 292–298. https://doi.org/10.2307/3543686.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Systematic Biology*, *59*(3), 307–321. https://doi.org/10.1093/sysbio/syq010.

Handschumacher, L., Steinarsdóttir, M. B., Edmands, S., & Ingólfsson, A. (2010). Phylogeography of the rock pool copepod *Tigriopus brevicornis* (Harpacticoida) in the northern North Atlantic, and its relationship to other species of the genus. *Marine Biology*, *157*(6), 1357–1366. https://doi.org/10.1007/s00227-010-1415-7.

ICZN - International Commission on Zoological Nomenclature. (1999). *International Code of Zoological Nomenclature*. 4th ed. International Trust for Zoological Nomenclature; 306 pp.

Incagnone, G., Marrone, F., Barone, R., Robba, L., & Naselli-Flores, L. (2015). How do freshwater organisms cross the "dry ocean"? A review on passive dispersal and colonization processes with a special focus on temporary ponds. *Hydrobiologia*, *750*(1), 103–123. https://doi.org/10.1007/s10750-014-2110-3.

Issel, R. (1914). Vita latente per concentrazione dell'acqua (anabiosi osmotica) e biologia delle pozze di scogliera. *Station Zu Mittheilungen Aus Der Zoologischen Station Zu Neapel*, *22*, 191–255.

Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., & Flouri, T. (2017). Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics*, *33*(11), 1630–1638. https://doi.org/10.1093/bioinformatics/btx025.

Ki, J. S., Lee, K. W., Park, H. G., Chullasorn, S., Dahms, H. U., & Lee, J. S. (2009). Phylogeography of the copepod *Tigriopus japonicus* along the Northwest Pacific rim. *Journal of Plankton Research*, *31*(2), 209–221. https://doi.org/10.1093/plankt/fbn100.

Korn, M., & Hundsdoerfer, A. K. (2016). Molecular phylogeny, morphology and taxonomy of Moroccan *Triops granarius* (Lucas, 1864) (Crustacea: Notostraca), with the description of two new species. *Zootaxa*, *4178*(3), 328–346. https://doi.org/10.11646/zootaxa.4178.3.2.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*(6), 1695–1701.

Lang, K. (1948). *Monographie der Harpacticiden I+ II*, *reprint*. Otto Koeltz Science Publishers.

Machida, R. J., Miya, M. U., Nishida, M., & Nishida, S. (2002). Complete mitochondrial DNA sequence of Tigriopus japonicus (Crustacea: Copepoda). *Marine Biotechnology*, *4*(4), 406–417. https://doi.org/10.1007/s10126-002-0033-x.

Marrone, F., Vecchioni, L., Deidun, A., Mabrouki, Y., Abdeslam, A., & Arculeo, M. (2020). DNA taxonomy of the potamid freshwater crabs from Northern Africa (Decapoda, Potamidae). *Zoologica Scripta*, *49*, 473–487. https://doi.org/10.1111/zsc.12415.

Mastrantonio, V., Porretta, D., Bellini, R., Nascetti, G., & Urbanelli, S. (2015). Molecular systematics and origin of the Mediterranean Sea rock pool mosquitoes of the *Aedes mariae* (Diptera: Culicidae) complex. *Annals of the Entomological Society of America*, *108*(4), 593–599. https://doi.org/10.1093/aesa/sav031.

McAllen, R. (1999). *Enteromorpha intestinalis* a refuge for the supralittoral rockpool harpacticoid copepod *Tigriopus brevicornis*. *Journal of the Marine Biological Association of the United Kingdom*, *79*(6), 1125–1126. https://doi.org/10.1017/S0025315499001393.

Mistakidis, M. (1949). A new variety of *Tigriopus lilljeborgii* Norman. *Dove Marine Laboratory Report*, *10*(3), 55–70.

Monard, A. (1935). Note préliminaire sur la faune des Harpacticoïdes marins d'Alger. *Bulletin De La Station D'aquiculture Et De Pêche Castiglione*, *1935*(1), 45–85.

Müller, O. F. (1776). *Zoologiae Danicae prodromus: seu Animalium Daniae et Norvegiae indigenarum characteres, nomina, et synonyma imprimis popularium*. Typis Hallageriis. https://doi.org/10.5962/bhl.title.63795.

Norman, A. M. (1869). Shetland final dredging report. Part II. On the Crustacea, Tunicata, Polyzoa, Echinodermata, Actinozoa, Hydrozoa, and Porifera. *Report of the British Association for the Advancement of Science*, *38*, 247–336.

Ortman, B. D. (2008). *DNA barcoding the Medusozoa and Ctenophora. Doctoral Dissertations (AAI3345202)*. University of Connecticut.

Park, E. O., Lee, S., Cho, M., Yoon, S. H., Lee, Y., & Lee, W. (2014). A new species of the genus *Tigriopus* (Copepoda: Harpacticoida: Harpacticidae) from Antarctica. *Proceedings of the Biological Society of Washington*, *127*(1), 138–154. https://doi.org/10.2988/0006-324X-127.1.138.

Powlik, J. J. (1999). Habitat characters of *Tigriopus californicus* (Copepoda: Harpacticoida), with notes on the dispersal of supralittoral fauna. *Journal of the Marine Biological Association of the United Kingdom*, *79*(1), 85–92. https://doi.org/10.1017/S0025315498000095.

Powlik, J. J., & Lewis, A. G. (1996). Desiccation Resistance in *Tigriopus californicus* (Copepoda, Harpacticoida). *Estuarine, Coastal and Shelf Science*, *43*(4), 521–532. https://doi.org/10.1006/ecss.1996.0085.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Molecular Ecology*, *21*(8), 1864–1877. https://doi.org/10.1111/j.1365-294X.2011.05239.x.

QGIS Development Team (2016). *QGIS Geographic Information System. Open Source Geospatial Foundation Project*. Retrieved from: http://www.qgis.org/.

Ranade, M. R. (1957). Observations on the resistance of *Tigriopus fulvus* (Fischer) to changes in temperature and salinity. *Journal of the Marine Biological Association of the United Kingdom*, *36*(1), 115–119. https://doi.org/10.1017/S0025315400017112.

Richterich, P. (1998). Estimation of errors in "raw" DNA sequences: a validation study. *Genome Research*, *8*(3), 251–259. https://doi.org/10.1101/gr.8.3.251.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539–542. https://doi.org/10.1093/sysbio/sys029.

Sars, G. O. (1911). *An Account of the Crustacea of Norway: With Short Descriptions and Figures of All the Species. Copepoda. Harpacticoida*. Text. Copepoda. Harpacticoida. Plates. Bergen Museum.

Spöri, Y., & Flot, J. F. (2020). HaplowebMaker and CoMa: two web tools to delimit species using haplowebs and conspecificity matrices. *Methods in Ecology and Evolution*, *11*, 1434–1438. https://doi.org/10.1111/2041-210X.13454.

Strøm, H. (1765). Beskrivelse over Ti Norske Insecter. Det Kiöbenhavn Selfkabs Skrifter. *Acta Hafniensia*, *9*, 572–595.

Swanson, G. A. (1984). Dissemination of amphipods by waterfowl. *The Journal of Wildlife Management*, *48*(3), 988–991. https://doi.org/10.2307/3801453.

Swofford, D. L. (2002). *Phylogenetic analysis using parsimony (*and Other Methods). Version 4*. Sinauer Associates. doi: https://doi.org/10.1007/978-1-4020-6754-9_12413.

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673–4680. https://doi.org/10.1093/nar/22.22.4673.

Underwood, A. J., & Skilleter, G. A. (1996). Effects of patch-size on the structure of assemblages in rock pools. *The Journal of Experimental Marine Biology and Ecology*, *197*(1), 63–90. https://doi.org/10.1016/0022-0981(95)00145-X.

Van Douwe, C. (1913). *Tigriopus fulvus* Fisher, var. *adriatica*, ein typischer Rock pools-Copepode. *Biologisches Zentralblatt*, *33*, 256–258.

Vecchioni, L., Marrone, F., Rodilla, M., Belda, E. J., & Arculeo, M. (2019). An account on the taxonomy and molecular diversity of a Mediterranean rock pool-dwelling harpacticoid copepod, *Tigriopus fulvus* (Fischer, 1860) (Copepoda, Harpacticoida). *Ciencias Marinas*, *45*(2), 59–75. https://doi.org/10.7773/cm.v45i2.2946.

Vittor, B. A. (1971). *Effects of the environment on fitness related life history characters in Tigriopus californicus (Doctoral dissertation, Thesis (Ph. D.) - Oregon, Dept. of Biology)*.

von Reumont, B. M., Meusemann, K., Szucsich, N. U., Dell'Ampio, E., Gowri-Shankar, V., Bartel, D., & Wägele, J. W. (2009). Can comprehensive background knowledge be incorporated into substitution models to improve phylogenetic analyses? A case study on major arthropod relationships. *BMC Evolutionary Biology*, *9*(1), 119. https://doi.org/10.1186/1471-2148-9-119.

Walter, T. C., & Boxshall, G. (2020). *World of Copepods database*. Tigriopus fulvus *(Fischer, 1860)*. Accessed through: World Register of Marine Species at: http://www.marinespecies.org/aphia.php?p=taxdetails&id=116184 on 2020-06-12.

Wells, J. B. J. (2007). An annotated checklist and keys to the species of Copepoda Harpacticoida (Crustacea). *Zootaxa*, *1568*(1), 1–872. https://doi.org/10.11646/zootaxa.1568.1.1.

Williams, D. D. (2006). *The biology of temporary waters*. Oxford University Press.

Zhang, J., Kapli, P., Pavlidis, P., & Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, *29*(22), 2869–2876. https://doi.org/10.1093/bioinformatics/btt499.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Figure S1. Results of the ABGD analysis using distances based on the K2p model, and the *12S* mtDNA fragment.

Figure S2. Putative species singled out by the mPTP model based on the mtDNA *12S* fragment.

Figure S3. Restricted *locus typicus* of *Tigriopus fulvus*. Supra-tidal rock pool where a sample of *Tigriopus fulvus* collected in Farrobo in the municipality of Seixal, Madeira, Portugal.

Alignment S1. Alignment of all *Tigriopus* spp. *12S* sequences included in the study.

Alignment S2. Alignment of all *Tigriopus* spp. *28S* sequences included in the study.

Alignment S3. Alignment of *12S/28S* sequences included in the mito-nuclear concatenated phylogenetic tree.

How to cite this article: Vecchioni L, Arculeo M, Cottarelli V, Marrone F. Range-wide phylogeography and taxonomy of the marine rock pools dweller *Tigriopus fulvus* (Fischer, 1860) (Copepoda, Harpacticoida). *J Zool Syst Evol Res*. 2021;59:839–857. https://doi.org/10.1111/jzs.12457