Rediscovery of *Knipowitschia goerneri* and its molecular relationships with other European northern Mediterranean *Knipowitschia* species (Teleostei: Gobiidae)

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The Corfu dwarf goby, *Knipowitschia goerneri*, known only from the Korission Lagoon catchment on Kerkyra Island (Greece), was described in 1991 on the only existing material, consisting of five specimens. It has not been observed since 1983, and was thought to be possibly extinct. In 2014, we collected nine specimens of *K. goerneri* from Korission Lagoon. We found this species to be morphologically and genetically well distinguishable from all other *Knipowitschia* species. Analyses of the mitochondrial gene cytochrome b placed it as a sister species of *K. milleri*, which is another goby from the northern Ionian Sea basin.

Introduction

A renewed interest in the European fish fauna has resulted in the recent discovery or recognition of many new species, especially from the Mediterranean region (e. g., Freyhof et al., 2005; Kovačić, 2005; Kottelat, 2007; Miller & Šanda 2008; Buj et al., 2010). This is an area with an exceptionally rich freshwater ichtyofauna (Freyhof & Brooks, 2011), and the number of species continues to increase (e. g., Buj et al., 2014; Freyhof et al., 2014). Many freshwater fish species in the region are endemic to a single or a few neighbouring rivers, and are under threat from human activities (Smith & Darwall, 2006; Freyhof & Brooks, 2011).

Several fish species from the Mediterranean area are currently considered extinct (Smith & Darwall, 2006; Geiger et al., 2014). However, it is possible that some of these have been overlooked due to their cryptic behaviour, small size, absence of real search effort, lack of interest, political or social situation, or administrative obstruction.

One of the endemic species with an unclear conservation, as well as taxonomic status, is the Corfu dwarf goby, *Knipowitschia goerneri* Ahnelt, 1991. Known only from the Korission Lagoon

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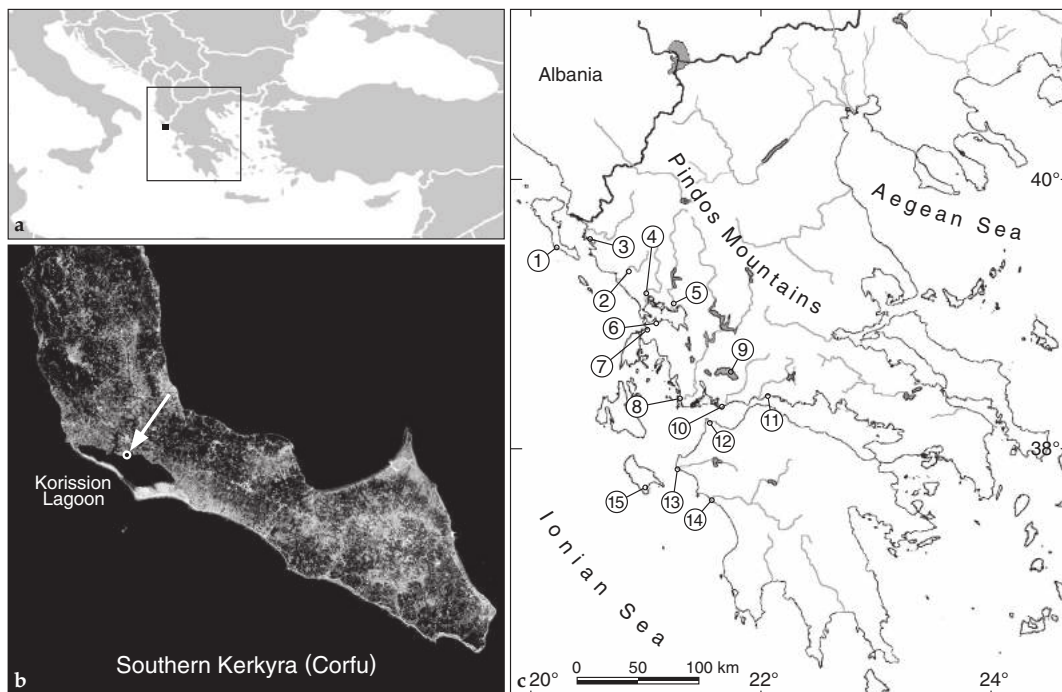   E-mail: rsanda@seznam.cz

Fig. 1. a, Location of Korission Lagoon, locality of *Knipowitschia goerneri*; **b,** sampling site at Korission Lagoon (indicated by arrow); **c,** Localities from where populations of *Knipowitschia* have been recorded in Greek Ionian Sea drainage (Economou et al., 2007; Barbieri et al., 2015): **1,** Korission Lagoon on Kerkyra Island (*K. goerneri*); **2,** Acheron River (*K. milleri*); **3–15,** still unidentified populations: **3,** Kalamas delta; **4,** Louros River; **5,** Arachthos River; **6,** Vlychos spring; **7,** Voulkaria Lake; **8,** Acheloos delta; **9,** Trichonis Lake; **10,** Evinos delta; **11,** Mornos delta; **12,** Prokopos-Kotychi Lagoons; **13,** Pinios River on Peloponnese; **14,** Alfios delta; and **15,** Zakynthos Island.

catchment on Kerkyra Island (Corfu), Greece (Ahnelt, 1991), this goby was described from the only existing material (five specimens) collected in 1968 and 1983, and taken from a freshwater spring and the brackish coastal lagoon, respectively. It was not observed in nature after 1983 (Kottelat & Freyhof, 2007), although at least one ichthyological survey was conducted at various localities on Kerkyra Island since this time (Kalogianni et al., 2006), and *K. goerneri* was suggested to be extinct (Economidis & Chrysopolitou, 2009). In the IUCN red list, this goby is listed as data deficient (Crivelli, 2006), as it is one of the least studied freshwater fish species with practically nothing known about its biology or ecology.

During an ichthyological survey on Kerkyra Island in July 2014, several specimens of *Knipowitschia* were collected in Korission Lagoon (Fig. 1). The aim of this work is to investigate the taxonomic status of this population and to provide information on its molecular relationships with other *Knipowitschia* species known from the European northern Mediterranean region.

Materials and methods

Counts and measurements follow Miller (1988), as other recent descriptions of *Knipowitschia* species also use the same methodology (Ahnelt 1995, 2011; Kovačić, 2005; Kovačić & Šanda, 2007; Miller, 2009). The length of the specimens is presented as standard length, and the terminology of the cephalic sensory systems follows Sanzo (1911) and Miller (1972). All specimens were stained in a 2 % solution of Cyanine Blue in distilled water using a reversible staining method (Saruwatari et al., 1997) to more clearly visualise scales and the cephalic sensory systems.

Abbreviations: SL, standard length; COI, cytochrome c oxidase subunit 1. Collection codes: NMP, National Museum, Prague; NMW, Natur-

DNA extraction and PCR. DNA was extracted from fin tissue preserved in 96 % ethanol using JETQUICK Tissue DNA Spin Kit (GENOMED) following the manufacturer's instructions. Mitochondrial gene cytochrome b was analysed. The amplification primers used were GluF and ThrR (Machordom & Doadrio, 2001). PCR reactions, amplification protocol, and PCR product purification follow Šanda et al. (2008). Sequencing was carried out by the Macrogen Service Centre (Seoul, South Korea) using the amplification primers.

Molecular data analysis. Sequences were aligned manually and revised in BioEdit (v.7.0.9). The final alignment included 1119 base pairs of cytochrome b. *Pomatoschistus bathi* was used as the outgroup. From the Adriatic basin, *K. panizzae* and *K. montenegrina* were included in the analyses as the other two freshwater endemics from this area (*K. radovici* and *K. mrakovcici*) are genetically almost identical to *K. panizzae* (Geiger et al., 2014).

The best fitting model of nucleotide substitution was assigned using JModeltest 0.1.1 (Posada, 2008). Using Akaike information criterion (AIC), the GTR+I model was selected. Two different approaches were used: Maximum Likelihood and Bayesian inference. Maximum Likelihood phylogenetic analyses were performed using the BEST approach implemented in PhyML, version 3.0.1, which combines nearest neighbour interchanges (NNI) and subtree pruning and regrafting (SPR) algorithms to maximize tree likelihood (Guindon & Gascuel, 2003). Five random starting trees were used. Branch support was quantified by bootstrap analysis with 1000 random replicates.

A Bayesian analysis was conducted using MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001). Two runs, each consisting of four Monte Carlo Markov Chains, were run simultaneously for 1 000 000 generations with sampling trees every 100 generations. The first 20 % of trees were discarded as burn-in and the remaining trees were used to construct a 50 % majority-rule consensus tree. The posterior probabilities were used to indicate the branch supports in the final tree.

Genetic divergences between and within species of *Knipowitschia*, based on cytochrome b nucleotide sequences, were calculated in Mega 5.2 (Tamura et al., 2011) as uncorrected pairwise genetic distance (p-distances).

Results

Knipowitschia goerneri
(Fig. 2)

Material examined. NMP P6V142862–142869, 1 male, 17.0 mm SL, 7 females, 13.1–20.6 mm SL; PMR VP3359, 1 female, 18.1 mm SL; Greece: Kerkyra Island: Korission Lagoon, 39°27'02" N 19°54'20" E; R. Šanda & J. Vukić, 26 July 2014 (GenBank accession numbers: KT809439–KT809446). – NMW 90253:1, 1, paratype, male, 17.8 mm SL; Greece: Corfu (Kerkyra) Island: NW beach of Korission Lagoon; P. Keymar, Aug 1983.

Generic identification. The following combination of characters identify the specimens as belonging to a species of the genus *Knipowitschia*: 1) suborbital row *a* of sensory papillae present; 2) pelvic disc with anterior transverse membrane; 3) cheek with several suborbital transverse rows of sensory papillae; 4) anterior oculoscapular canal ending in interorbital area, with paired pores λ, or at doubled pore κ (*K. goerneri*), or head canals absent; 5) no perianal organ; and 6) oculoscapular row *tra* of sensory papillae not reaching close to suborbital row *b*.

Diagnosis. The genus *Knipowitschia* includes 16 species. There are no characters unique to *K. goerneri*. *Knipowitschia goerneri* is easily distinguished from *K. byblisia*, *K. ephesi*, *K. montenegrina*, *K. mrakovcici* and *K. thessala* by having the body squamation continuous from the axilla to the caudal part along the lateral midline (vs. body squamation not continuous). *Knipowitschia goerneri* differs from *K. milleri*, *K. ephesi* and *K. montenegrina* by the presence of at least some head canals (vs. head canals absent). *Knipowitschia goerneri* is most clearly distinguished from *K. radovici*, *K. cameliae*, *K. caucasica*, *K. caunosi*, *K. longecaudata* and *K. panizzae* by the absence of the preopercular head canal (vs. preopercular head canal present). *Knipowitschia goerneri* differs from *K. iljini*, *K. cameliae*, *K. caucasica*, *K. caunosi*, *K. longecaudata*, *K. panizzae* and *K. bergi* by having reduced squamation along the second dorsal fin, i. e. dorsal naked along second dorsal fin up to the base of the penultimate or of last articulated ray (vs. squamation along the second dorsal fin not reduced up to the base of the penultimate or of last articulated ray). *Knipowitschia goerneri* differs from *K. mermere* by having the base of the last ray of the second dorsal fin before the vertical through the base of the last anal-fin ray (vs. last

ray of second dorsal fin at vertical of the base of the last anal-fin ray). *Knipowitschia goerneri* further differs from *K. mermere* by having a wider head (head width 17.9–22.3 % SL vs. 11.9–14.9; 58–69 % HL vs. 38–48) and a shorter snout (snout length 16–21 % HL vs. 22–31).

Description. Body moderately elongate, cross section circular anteriorly, compressed towards caudal peduncle. Head large and moderately depressed. Snout oblique, relatively longer in smaller specimens, shorter in large specimens. Eye large, dorsolateral, eye contour just slightly above dorsal profile, i. e. above snout and predorsal area. Interorbital space distinctly smaller than eye diameter and moderately narrow. Anterior nostril short, tubular, erect, without process from rim; posterior nostril pore-like, near orbit. Mouth oblique, with lower jaw slightly projecting forward to upper jaw, posterior angle of jaws below pupil. Branchiostegal membrane attached along entire lateral margin of isthmus. Body proportions given in Table 1.

Fins. First dorsal VI; second dorsal I/7–8; anal I/7–8; caudal 13 branched rays, 15–16 segmented; pectoral 15–16; pelvic I/5+5/I. First dorsal fin arising behind pectoral-fin base. First dorsal-fin spines III–V when depressed extending to second dorsal fin I in adult male, not reaching second dorsal in female. Interdorsal space distinct. Second dorsal fin commencing over or immediately behind vertical of anus, with last ray before vertical of last anal-fin ray. Anal fin commencing below second to third articulated ray of second dorsal fin. Caudal fin rounded. Pectoral fin extending

Table 1. Standard length and proportional measurements of *Knipowitschia goerneri* in this study and of type material (Ahnelt, 1991). Morphometric ratios from Ahnelt (1991) were recalculated as percentages.

	present research			type material
	range	mean	SD	range
Standard length (mm)	13.1–20.6			17.8–21.8
In percent of standard length				
Head length	30.0–33.8	31.7	1.3	30.0–34.2
Head width	19.1–22.3	20.3	1.1	17.9–20.0
Distance from snout to origin of first dorsal fin	39.2–42.2	41.2	1.0	39.1–43.9
Distance from snout to origin of second dorsal fin	56.1–61.7	59.1	2.0	54.6–61.3
Distance from snout to vertical of anus	55.2–59.7	57.1	1.4	55.2–61.3
Distance from snout to vertical of anal-fin origin	59.1–65.5	61.5	1.9	61.7–66.2
Distance from snout to vertical of pelvic-fin origin	31.2–35.9	32.9	1.5	30.3–34.5
Caudal-peduncle length	24.6–27.5	26.1	0.9	23.6–27.9
First dorsal-fin base	9.2–14.1	10.9	1.7	10.7–13.8
Second dorsal-fin base	13.0–16.0	14.9	1.1	16.1–18.3
Anal-fin base	13.5–17.1	15.2	1.2	16.1–17.9
Caudal-fin length	21.5–25.2	23.1	1.3	24.3–27.9
Pectoral-fin length	18.3–22.8	20.3	1.5	22.6–25.3
Pelvic-fin length	18.3–24.8	21.1	2.1	–
Body depth at pelvic-fin origin	21.0–23.8	22.7	1.1	20.7–23.9
Body depth at anal-fin origin	15.2–19.6	16.4	1.4	15.6–17.9
Body width at anal-fin origin	8.4–11.7	10.0	1.0	–
Caudal-peduncle depth	10.1–11.6	10.8	0.6	9.2–11.4
Distance from pelvic-fin origin to anus	22.5–30.4	25.9	2.4	–
In percent of head length				
Snout length	17–21	19.2	1.3	16–20
Eye diameter	21–25	23.4	1.5	17–27
Postorbital length	49–61	54.9	3.7	53–61
Head width	58–69	63.9	3.6	59–61
Ratios				
Caudal-peduncle depth in percent of its length	38–47	41.5	2.8	36–51
Interorbital width in percent of eye diameter	42–55	47.2	5.2	39–54
Pelvic-fin length in percent of distance from pelvic origin to anus	74–94	81.9	6.2	79–98

Fig. 2. *Knipowitschia goerneri*, NMP P6V142865, 17.0 mm SL, male, and NMP P6V142867, 18.9 mm SL, female; Greece: Kerkyra Island: Korission Lagoon.

back to below posterior end of first dorsal fin. Pelvic disc rounded, nearly reaching anus in male, shorter in female; anterior membrane in midline ⅓ to ½ of pelvic spine length, free edge of anterior membrane smooth. Lengths of fins and fin bases in proportion to standard body length given in Table 1.

Scales. Side of body and caudal peduncle covered with ctenoid scales; predorsal and dorsal region, back to base of penultimate or of last articulated ray of second dorsal fin naked, breast and belly naked; 30 to 33 in lateral series. Body squamation continuous from axilla to caudal part along lateral midline. Upper edge of scaled area extending from behind upper part of pectoral axilla backwards and up, reaching second dorsal fin at its posterior end. Lower edge of scaled area extending from behind pectoral-axilla bottom backwards and up to vertical of anus and interdorsal space, and then downwards to anal fin, variably reaching it. Narrowest section of scaled area along lateral midline between interdorsal space and anus.

Coloration. Preserved specimens. Body dark brown above and whitish below; male darker than female. Body, except most of underside in female and belly in male, covered with numerous melanophores, variably and irregularly mottled with hardly recognizable patterns. Five pale dorsal saddles: at nape opposite opercle, origin of first dorsal fin, interdorsal space and origin

of second dorsal fin, posterior end of second dorsal fin and posterior part of caudal peduncle. In female several darker blotches recognizable along lateral midline. Entire head pigmented in male, in female underside mostly whitish and poorly distinguished preorbital bars extending to form a Y-shaped blotch on chin. First dorsal fin in adult female with pigmentation anteriorly at base, oblique transparent band in lower half above pigmented area at base. Upper half of fin with broad dark oblique band above transparent band and transparent upper edge at fin-spine tips. First dorsal fin in adult male entirely pigmented, with more intensive black spot in mid-height of fin between spine IV and V. Second dorsal fin and caudal fin pigmented without recognizable pattern. Pectoral fin with dark blotch on upper-ray origin and rarely scattered melanophores on rest of upper part of fin. Pelvic fin whitish in female, pigmented and covered with melanophores in male.

Lateral-line system. Cephalic canals present. Anterior oculoscapular canal present, but much reduced, divided into two or three parts: one behind eye from pore κ (always paired) to pore α; second more posteriorly from pore α' to pore ρ; third short canal with pores λ and λ' between eyes of variable occurrence in specimens. Posterior oculoscapular and preopercular canals always absent. Rows of sensory papillae: Preorbital with three median rows and two rows lateral to
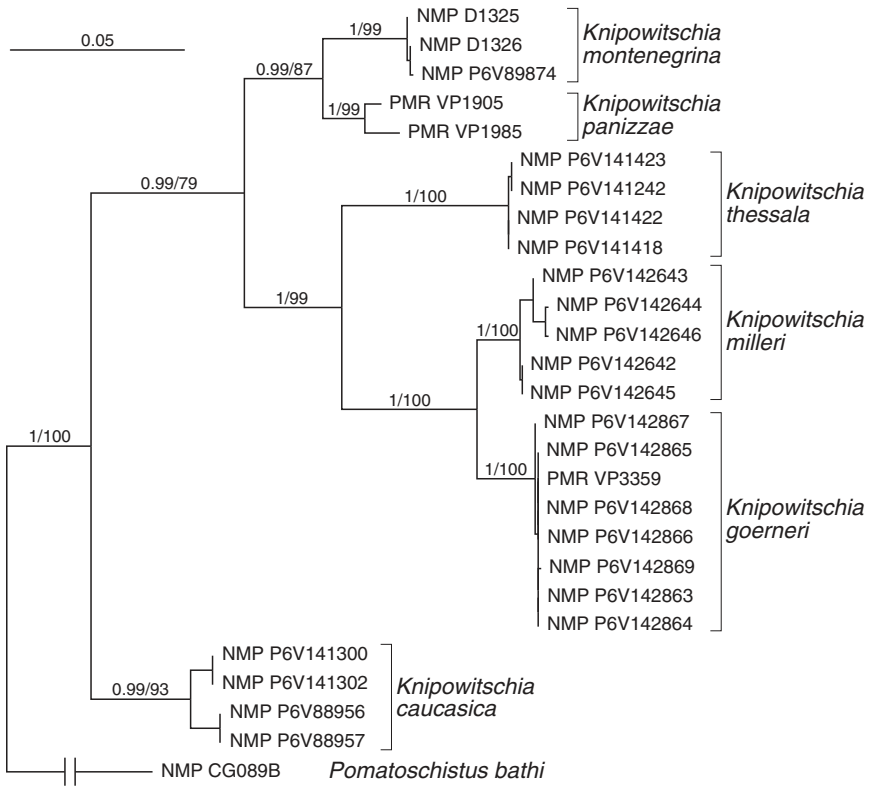
Fig. 3. Maximum Likelihood estimation of phylogenetic relationships of analysed *Knipowitschia* species based on cytochrome b nucleotide sequences. Numbers on branches are posterior probabilities / bootstrap support of nodes.

nostrils. Suborbital rows with infraorbital row *a* extending forward to below anterior part or anterior edge of pupil, posteriorly proliferated in transverse row *atp* and a few shorter transverse rows of 2–3 papillae. Longitudinal rows *b* not extending anteriorly before vertical of posterior border of orbit. Six or seven transverse *c* rows. Longitudinal row *d* with continuous supralabial and cheek parts. Three preopercular mandibular rows: external row *e* and internal row *i*, both divided, and mental row *f*. Oculoscapular rows *tra*, *z*, x^1, *trp*, x^2, *y* present, with x^1 divided by row *trp* in anterior and posterior parts, additional papillae present at position of missing preopercular canal (below level of oculoscapular row *z* and slightly in front of its vertical), and single papilla present below posterior part of x^1. Axillary rows as^1, la^1, as^2, la^2 and as^3 visible. Opercle with transverse row *ot*, superior longitudinal row *os* and inferior longitudinal row *oi*. Anterior dorsal row *n* transversal, rows *g* and *h* longitudinal, rows *o* and *m* absent. Interorbital longitudinal row *p* present.

Molecular relationships. Both Maximum Likelihood and Bayesian inference analyses resulted in an identical tree topology with a high statistical support of branching (Fig. 3). Our results based on cytochrome b nucleotide sequence analyses show that *K. goerneri* is a sister species of *K. milleri*, the species of *Knipowitschia* from the geographically closest locality. The mean genetic divergence between cytochrome b nucleotide sequences of these two species is 3.2 % (Table 2), and they form reciprocally monophyletic lineages (Fig. 3).

Discussion

Morphology. Our morphological examination confirmed that the newly collected *Knipowitschia* from Korission Lagoon is *K. goerneri*. This species cannot be confused with any other known goby species from the European coast of the Mediterranean, including *K. milleri* (Ahnelt & Bianco, 1990), the only other *Knipowitschia* species from the

north-eastern part of the Ionian Sea basin. Unlike *K. goerneri*, *K. milleri* completely lacks head canals. The degree of reduction of both body squamation and head canals in *K. goerneri* are unique among all 16 named *Knipowitschia* species (Ahnelt, 1991, 1995, 2011; Miller, 2004a, 2009; Kovačić, 2005: table 2; Kottelat & Freyhof, 2007; Kovačić & Šanda, 2007; Geiger et al., 2014) with the only exception being *K. mermere* Ahnelt, 1995, a species from Anatolia (Ahnelt, 1995) located so far away that the presence of *K. mermere* in Korission Lagoon is highly improbable. In addition, the values of some morphometric characters of *K. goerneri* and *K. mermere* do not overlap (head width, snout length; see diagnosis), or overlap only slightly (caudal-fin length 21.5–27.9 % SL, vs. 18.1–22.0; body depth at pelvic-fin origin 20.7–23.9 % SL, vs. 19.2–21.6) (see Table 1 and Ahnelt, 1991, 1995). Furthermore, *K. mermere* is a freshwater species, which is related to *K. thessala* (Vinciguerra, 1921) and is considerably divergent from *K. milleri*, based on COI gene nucleotide sequence analysis (Geiger et al., 2014), while *K. goerneri* is closely related to *K. milleri*, based on cytochrome b gene nucleotide sequence analyses (this study).

Comparison with the type material of *K. goerneri* showed that the morphology of the newly collected specimens matches well with the original species description (Ahnelt, 1991). Two extensions of the original meristic character ranges were observed in the new material of *K. goerneri*, which is probably a result of the smaller number of individuals used for the original description. First, we observed both the second dorsal fin and anal fin to have I/7–8, vs. I/8 for both fins in the original description. Second, one specimen exhibited 30 scales in the lateral series, vs. a range of 32–33 in the original description. It was also observed in the recent specimens that the pelvic disc of a single male nearly reached the anus, while in the original description the pelvic disc of both males reached the anus.

The reduction of the cephalic lateral line canals in the newly collected specimens of *K. goerneri* matches with two types of reduction observed by Ahnelt, 1991 (the types shown in his figures 3a,d and 3b,e), both of which are well-represented in the present sample. Some morphological details missing in the original description were provided in the present data (e. g. caudal-fin segmented-ray count, exact limits of scaled area on body surface). Ahnelt (1991) described the species based on only five specimens, of which two were males and

three females. Measurements conducted on the slightly larger sample size and better preserved, freshly collected specimens, showed somewhat higher morphological variability than previously thought (Table 1; Ahnelt, 1991: table 1). However, some morphometric characters (fin bases and fin lengths) in the present study largely extend one side of the range of values compared with the original description. This is probably a result of growth allometry, as five of the nine specimens used in this study were small (13.1–15.7 mm SL) compared to the comparatively larger specimens used in the original description (17.8–21.8 mm SL) (Ahnelt, 1991). The five new small specimens had, proportionally to the standard length, shorter dorsal- and anal-fin bases, as well as shorter caudal- and pectoral-fin lengths compared with the larger specimens in our sample and in the type material.

Molecular relationships. Based on cytochrome b nucleotide sequence analyses, *K. goerneri* is closely related to *K. milleri*, another species from the north-eastern part of the Ionian Sea basin (Fig. 3). The cytochrome b haplotypes of both species form unambiguous reciprocally monophyletic lineages (Fig. 3), and together they form a well-supported clade, which is in a sister position to the cytochrome b haplotypes of *K. thessala*, a species endemic to the Thessalian Pinios River drainage in the north-western Aegean Sea basin (on the other side of the Pindos Range, a major biogeographic barrier). The genetic distance between *K. goerneri* and *K. milleri* inferred from the cytochrome b gene sequences is around 3 % (Table 2). The intraspecific genetic variability of both species based on cytochrome b is several times lower; in *K. milleri* it does not exceed 0.9 %, whereas in *K. goerneri* it is much lower, with a maximum of 0.2 %.

Several other populations of *Knipowitschia*, whose taxonomic status is not clarified, are known from the north-eastern Ionian Sea basin (from the Kalamas River to the western Peloponnese Alfios River; Economou et al., 2007, Barbieri et al., 2015; Fig. 1). Only some of these have been genetically studied thus far (Vanhove et al., 2012; Geiger et al., 2014). These studies show that several *Knipowitschia* populations (from the Acheloos and Evinos River basins and the Prokopos-Kotychi drainage in north-western Peloponnese) are closely related to *K. milleri*. Each study used different mitochondrial genetic markers (12S and 16S in Vanhove et al., 2012; COI in Geiger et al.,

2014; cytochrome b in our study), thus it is not possible to compare the results directly due to the different mutational rates in different genes (Wan et al., 2004). As *K. goerneri* is also closely related to *K. milleri*, it is necessary to compare all populations of *Knipowitschia* from the north-eastern Ionian Sea basin with these two species in order to delimit their exact distribution range and to clarify the taxonomy of the *Knipowitschia* populations from this area. This should ideally be done by combining the genetic and morphological approaches, and could potentially result in the discovery of additional species.

Ecology and distribution. *Knipowitschia goerneri* was originally found in a freshwater spring, as well as in the brackish lagoon of Korission (Ah-nelt, 1991). We discovered that the species is still present in the lagoon, though we sampled at two sites and the species was only found at one. The sampling point at the lagoon was very shallow (maximum depth of 40 cm), with a muddy bottom containing numerous shell remains and a dense filamentous algal covering. From the locality where it was found, we assume that *K. goerneri* is a euryhaline and eurythermal species. It is probable that the conditions in the lagoon vary substantially during the course of the year. The size and status of the population are unknown. Further research of the Korission Lagoon catchment and its surroundings on Kerkyra Island is necessary in order to characterise the ecological and biological requirements of *K. goerneri*, its habitat preferences and its distribution. This species may occur in other wetland sites on the island, as well as on the mainland opposite Kerkyra in Greece and in Albania.

Conservation and threats. Ahnelt (1991) de-scribed *K. goerneri* based on specimens from a freshwater spring south-west of Korission Lagoon (the type locality), as well as the north-west beach of the lagoon. Since the work of Ahnelt (1991), the lagoon has not been mentioned as the locality for *K. goerneri* (Economidis, 1995; Miller, 2004b; Economidis & Chrysopolitou, 2009), and there is no published evidence that it has been investigated for its presence since 1983. The assumption that this species is critically endangered or possibly extinct (Miller, 2004b; Economidis & Chrysopolitou, 2009) was based on the destruction of Gardiki Spring, situated north of the lagoon (Economidis, 1995), which was erroneously mentioned as the only locality of *K. goerneri* (Miller, 2004b; Economidis & Chrysopolitou, 2009), even though the species has never been found there. Although *K. goerneri* was listed in the Red Data Book of Greece as critically endangered and possibly extinct (Economidis & Chrysopolitou, 2009), no conservation actions have been proposed for this species (Economidis & Chrysopolitou, 2009). Despite the Korission Lagoon catchment being designated as a Natura 2000 site (European Environment Agency, 2014), it is threatened by development, pollution, fisheries, and over-pumping groundwater.

Conclusions. The rediscovery of *K. goerneri* in this study, as well as another freshwater fish species, *Telestes miloradi* (Jelić & Jelić, 2015), shows that even in Europe species can be overlooked for decades. Hence, it is possible that some other species currently thought to be extinct (Smith & Darwal, 2006; Kottelat & Freyhof, 2007; Geiger et al., 2014) could in fact still persist (e. g. *K. cameliae*, *Chondrostoma scodrense*). Intensive field work is

Table 2. Mean uncorrected pairwise genetic distance (p-distance; %) between analysed species of *Knipowitschia* based on cytochrome b nucleotide sequence analysis. Number of analysed specimens and range of intraspecific genetic variability included next to species names in parentheses, and mean intraspecific genetic variability included in bold font.

	K. goerneri	K. milleri	K. thessala	K. montengrina	K. panizzae	K. caucasica	P. bathi
K. goerneri (8; 0–0.18)	**0.045**						
K. milleri (5; 0–0.89)	3.2	**0.55**					
K. thessala (4; 0–0.09)	7.8	7.6	**0.06**				
K. montenegrina (3; 0.09–0.18)	8.2	8.8	8.6	**0.12**			
K. panizzae (2; –)	8.5	8.9	8.1	3.9	**1.43**		
K. caucasica (4; 0–1.43)	10.3	10.1	9.9	8.7	8.5	**0.95**	
Pomatoschistus bathi (1; –)	16.4	17.1	16.8	16.3	16.2	15.2	–

needed to better evaluate the possible presence of such taxa in the Mediterranean region.

Comparative material. *Knipowitschia milleri*: PMR VP3212, 1 female, 22.9 mm SL; PMR VP3213, 1 male, 20.2 mm SL; PMR VP3214, 1 juvenile, 17.5 mm SL; Greece: Acheron River.

Material used in molecular genetic analysis: *K. milleri*: NMP P6V142642–142646, 5; Greece: Acheron River, 39°14'34"N 20°28'40"E (GenBank accession numbers: KT809434–KT809438).

K. thessala: NMP P6V141242, 1; Greece: Pinios drainage: Enipeas River, 39°33'43"N 22°4'45"E (GenBank accession number: KT809433). – NMP P6V141418, 1; NMP 141422–141423, 2, Greece: Pinios drainage: Litheos River, 39°33'14"N 21°46'09"E (GenBank accession numbers: KF214247, KT809431–KT809432).

K. montenegrina: NMP P6V89874, 1; NMP D1326–D1325, 2; Montenegro: Morača River, 42°18'37"N 19°11'56"E (GenBank accession numbers: KT809450–KT809452).

K. panizzae: PMR VP1905, 1; Croatia: Privlaka, 44°16'37"N 15°07'53"E (GenBank accession number: KT809453). – PMR VP1985, 1; Croatia: Cetina River, 43°26'21"N 16°45'02"E (GenBank accession number: KT809454).

K. caucasica: NMP P6V88956–88957, 2; Bulgaria: Pomorijsko Lake, 42°33'56"N 27°37'56"E (GenBank accession number: KF214250, KT809449). – NMP P6V141300, 1; NMP 141302, 1; Greece: Volvi Lake, 40°39'14"N 23°30'04"E (GenBank accession number: KT809447–KT809448).

Pomatoschistus bathi: NMP CG089B, 1; Montenegro: Bečići: Adriatic Sea, 42°16'26"N 18°53'16"E (GenBank accession number: KT809430).

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