

A review of the *Tripterygion tripteronotus* (Risso, 1810) complex, with a description of a new species from the Mediterranean Sea (Teleostei: Tripterygiidae)

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SUMMARY: We compared specimens of *Tripterygion tripteronotus* from 52 localities of the Mediterranean Sea and adjacent waters, using four gene sequences (12S rRNA, tRNA-valine, 16S rRNA and COI) and morphological characters. Two well-differentiated clades with a mean genetic divergence of $6.89 \pm 0.73\%$ were found with molecular data, indicating the existence of two different species. These two species have disjunctive geographic distribution areas without any molecular hybrid populations. Subtle but diagnostic morphological differences were also present between the two species. *T. tripteronotus* is restricted to the northern Mediterranean basin, from the NE coast of Spain to Greece and Turkey, including the islands of Malta and Cyprus. *T. tartessicum* n. sp. is geographically distributed along the southern coast of Spain, from Cape of La Nao to the Gulf of Cadiz, the Balearic Islands and northern Africa, from Morocco to Tunisia. According to molecular data, these two species could have diverged during the Pliocene glaciations 2.7-3.6 Mya.

Keywords: *Tripterygion*, new species, molecular data, morphology, taxonomy, Mediterranean Sea.

RESUMEN: REVISIÓN DEL COMPLEJO *TRIPTERYGION TRIPTERONOTUS* (RISSE, 1810), Y DESCRIPCIÓN DE UNA NUEVA ESPECIE EN EL MAR MEDITERRÁNEO (TELEOSTEI: TRIPTERYGIIDAE). – Se han estudiado especímenes de *Tripterygion tripteronotus* procedentes de 52 localidades mediterráneas y de aguas atlánticas adyacentes, utilizando cuatro genes mitocondriales distintos (12S rRNA, tRNA-valine, 16S rRNA y COI) así como varios caracteres morfológicos. Se han encontrado dos grupos molecularmente bien diferenciados, la divergencia genética media presente entre ambos es de un $6.89 \pm 0.73\%$, lo que implica la presencia de dos especies distintas. Sus áreas de distribución están separadas y no se han encontrado poblaciones molecularmente híbridas. Además, se han encontrado pequeñas diferencias morfológicas que pueden ser utilizadas como caracteres diagnósticos entre las dos especies. *T. tripteronotus* se encuentra en la cuenca mediterránea norte, extendiéndose desde la costa NE de España hasta Grecia y Turquía, incluyendo las islas de Malta y Chipre. *T. tartessicum* n. sp. se extiende por la costa sur de España, desde Cabo La Nao hasta el Golfo de Cádiz, las islas Baleares y el norte de África, desde Marruecos a Túnez. De acuerdo con los datos moleculares obtenidos, ambas especies pudieron divergir durante las glaciaciones ocurridas en el Plioceno hace unos 2.7-3.6 m.a.

Palabras clave: *Tripterygion*, nueva especie, datos moleculares, morfología, taxonomía, Mar Mediterráneo.

INTRODUCTION

Molecular data provide a complementary approach to discriminate species separated by subtle morphological characters (Knowlton, 1993; Avise, 1994; Held and Wagele, 2005). In the last few years, numerous authors have used molecular methods to

detect cryptic species, either in fishes (Gilles *et al.*, 2000; Gysels *et al.*, 2004; Almada *et al.*, 2005a) or in other marine organisms (Tarjuelo *et al.*, 2001).

The family Tripterygiidae contains species of bottom-living blennioid fishes, usually associated with rocky habitats and inhabiting cold, temperate, subtropical and tropical areas (Fricke, 2002). The

genus *Tripterygion* Risso, 1826, is the only genus of the family Tripterygiidae in the Mediterranean Sea and on the northeastern Atlantic coast (Zander, 1986). Three species have been described: *T. tripteronotus*, Risso, 1810, and *T. melanurus*, Guichenot, 1845, are endemic to the Mediterranean, and *T. delaisi* Cadenat and Blache, 1971, is found in both areas (Wirtz, 1980). Individuals of the three species are common in shallow coastal waters, always living in rocky areas. *T. tripteronotus* inhabits light-exposed and shady biotopes preferably between 0 and 3 m, whereas *T. delaisi* uses similar biotopes but at greater depth (between 0 and 40 m) and also biotopes with reduced light such as under overhanging rocks or entrances to caves. Finally, *T. melanurus* inhabits walls or ceilings of marine caves and other dimly lit biotopes (Wirtz, 1978; Macpherson, 1994; Zander, 2004).

The species of the genus *Tripterygion* form a monophyletic group and each previously described species is well differentiated genetically (Carreras-Carbonell *et al.*, 2005). However, this recent phylogeographic study, using molecular data, indicated that: (1) the two morphotypes of *T. melanurus*, traditionally considered as two different subspecies by Zander (1986), were not genetically different with the markers used, although there may be differences on other parts of the DNA sequence, (2) the two currently accepted subspecies for *T. delaisi* (Zander, 1986) were molecularly validated, and (3) *T. tripteronotus*, considered at present as a single species, showed two well-defined and highly supported clades with greater divergence than that shown between the two *T. delaisi* subspecies, revealing the existence of two cryptic species within *T. tripteronotus* (Carreras-Carbonell *et al.*, 2005). Zander and Heymer (1970) had already described two different pattern bands in the caudal region for *T. tripteronotus* individuals from Banyuls-sur-Mer (France) and Mdiq (Morocco). Later, Zander and Heymer (1976) showed slight morphological differences in the dorsal fins between *T. tripteronotus* specimens from Israel and Lebanon in comparison with specimens from the northwestern Mediterranean. Although no taxonomic status was assigned, these morphological differences could be related to the two *T. tripteronotus* clades found by Carreras-Carbonell *et al.* (2005).

The aim of the present work was to describe the new species and search for morphological characters that allow the two species to be differentiated

using specimens from 52 localities of the Mediterranean Sea and adjacent waters.

MATERIALS AND METHODS

Sampling and repositories

Specimens of the two species of *Tripterygion* were collected at different localities of the Mediterranean Sea and the Gulf of Cadiz; specimens from the Staatliches Museum fuer Naturkunde (Stuttgart, SMNS) were also used, with the result that the individuals came from a total of 52 localities (Fig. 1). The number of individuals used for morphological and molecular analyses, as well as supplementary details about each sampling locality, are shown in Table 1.

The type series of the new species are deposited in the collections of the Instituto de Ciencias del Mar (Barcelona, IIPB), the Museo Nacional de Ciencias Naturales (Madrid, MNCN) and the Staatliches Museum fuer Naturkunde (Stuttgart, SMNS) (see Table 1).

Morphological analysis

In the description of the new species, the data of the paratypes follow those of the holotype, in parentheses. Lengths given and the terminology and other measurements used mainly follow Zander and Heymer (1970), Wheeler and Dunne (1975) and Fricke (1997). Lengths are explained below:

Predorsal length (PD) distance between middle of upper lip and base of the 1st spine of the first dorsal fin.

Head length (HL) distance between middle of upper lip and upper insertion of operculum.

Orbital diameter (OD) maximum eye diameter.

Preorbital length (PO) distance between middle of upper lip and anterior margin of eye.

The middle of the upper lip is used as the starting point for several lengths rather than the tip of the upper jaw, as the latter may be protractile.

Mandibular pore formula. This formula gives the number of pores under left dentary + number of median pore(s) + number of pores under right dentary.

Individuals were photographed alive in order to check their colour pattern; one or two right gills were removed and kept in absolute ethanol at room temperature. Specimens were individually fixed using buffered formol with 2% borax to maintain the colour pattern for further morphological analyses.

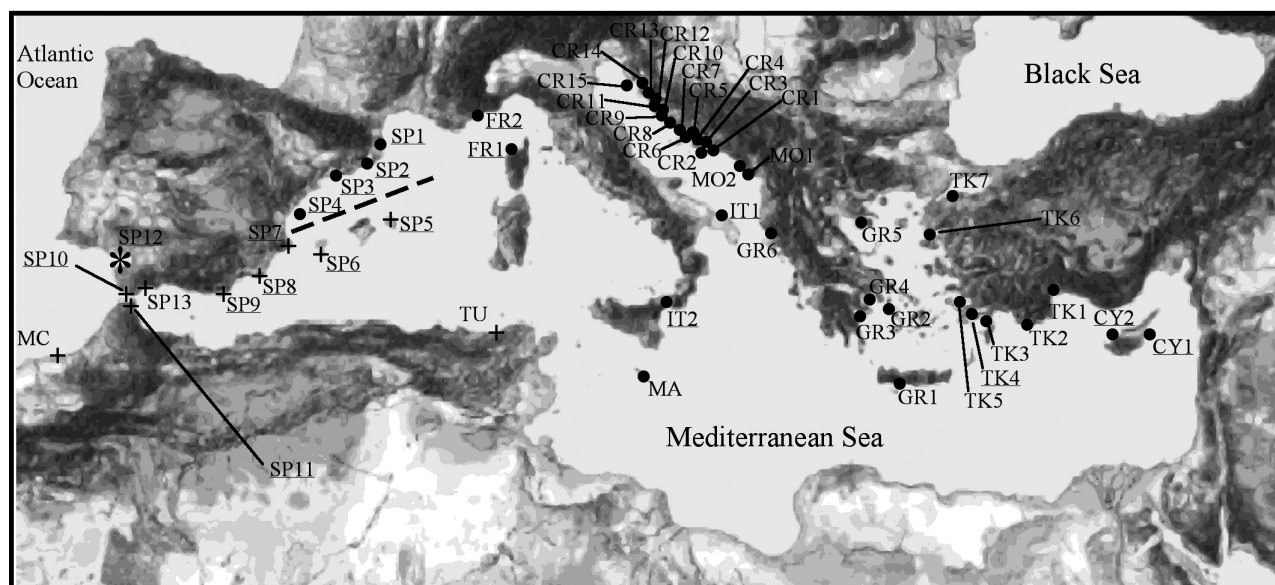


FIG. 1. – Sampling localities for *T. tripteronotus* (●) and *T. tartessicum* (+). Dashed line shows the break zone between the two species along the Spanish Mediterranean coast. (*): Holotype locality. Localities which individuals were molecularly analysed are underlined. See Table 1 for locality abbreviations and further details.

Key

The morphological taxonomic key only works for both sexes when morphometric measurements are used. Sometimes, males can also be distinguished by discrete morphological characters, while females are identifiable only by their geographical distribution and accompanying males.

Molecular analysis

In order to analyse the genetic difference between the two clades of *T. tripteronotus*, we used the sequences of the 12S, tRNA-valine and 16S (acc. num: AJ868510-23, AJ937970-74, AJ872149-60 and AJ937975-79), and COI (acc. num: AJ872128-40 and AJ937862-65) genes from Carreras-Carbonell *et al.* (2005). The same gene sequences were amplified from additional individuals from CY1 (AM260942 and AM260946), CY2 (AM260943 and AM260946), TK4 (AM260944 and AM260947), TK6 (AM260944 and AM260947), IT2 (AM260940-1 and AM260945), FR2 (AM086386-7), SP3 (AM086388-9), SP4 (AM086390-1), SP5 (AM086392-3), SP6 (AM086394-5), SP7 (AM086396-7) and SP8 (AM086398-9) (for location abbreviations and further sampling locality details see Table 1). We used *Tripterygion delaisi xanthosoma* (family Tripterygiidae) and *Parablennius rouxi* (family Blenniidae) from SP2 as internal and external out-

group species respectively (AJ868503, AJ872118 and AJ872164 for *T. d. xanthosoma* and AJ966656-62 for *P. rouxi*).

The homogeneity of base composition across taxa was assessed using the goodness-of-fit (χ^2) test and the incongruence length difference test (ILD) (Farris *et al.*, 1994) was computed to assess analytical differences between genes; both tests are implemented in PAUP* ver. 4.0b10 (Swofford, 2001). In the latter test only parsimony informative characters were included and heuristic searches were performed with 10 random stepwise additions with TBR branch swapping and 1000 randomisations. Furthermore, trees were considered significantly incongruent whenever different gene trees conflicted at nodes that were supported by BI posterior probabilities >95% (Moyer *et al.*, 2004).

Phylogenetic trees were inferred by Bayesian inference (BI) using Mr Bayes 3.0b4 (Huelsenbeck and Ronquist, 2001) because it seems to be the best methodology for inferring phylogenetic relationships between species (Alfaro *et al.*, 2003), and its reconstruction does not seem to be affected by saturated positions (Carreras-Carbonell *et al.*, 2005). The computer program MODELTEST ver. 3.06 (Posada and Crandall, 1998) was used to choose the best-fit ML model under the Akaike Information Criterion (AIC) for each gene separately and was later applied in the BI analyses. The MCMC (Markov chain Monte Carlo) algorithm with four Markov chains was run for 1500000 generations, sampled every 100 generations

TABLE 1. – Specimens of the two *Tripterygion* species collected at different localities of the Mediterranean and Atlantic adjacent waters. The number of individuals used for morphological (Nm) and molecular (Ng) analyses, for each locality, are detailed.

	Map code	Country	Locality	Latitude/ Longitude	Depth (m)	Collection date	Nm/ Ng	LT range (mm)	Catalogue number
<i>Tripterygion tripteronotus</i>	CY1	Cyprus	Akrotirion Gatas/Cape Greco, southeastern corner	34°32'N 33°00'E	0-1	May 2002	6/4	42-47	SMNS 23059
	CY2a	Cyprus	Karavas Alsavcak Bay, small island on eastern side of bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'13''N 33°13'15''E	0-1	20 May 1997	1/0	38	SMNS 19066
	CY2b	Cyprus	Karavas Alsavcak Bay, small island on eastern side of bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'13''N 33°13'06''E	0-1	23 May 1997	1/0	41	SMNS 19085
	CY2c	Cyprus	Karavas Alsavcak Bay, small island on eastern side of bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'13''N 33°13'06''E	0-1	24 May 1997	5/1	34-43	SMNS 19091
	CY2d	Cyprus	Karavas Alsavcak Bay, small island on eastern side of bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'13''N 33°13'06''E	0.6-1.5	27 May 1997	6/2	36-43	SMNS 19098
	CY2e	Cyprus	Karavas Alsavcak Bay, small island on eastern side of bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'13''N 33°13'06''E	3-5.5	27 May 1997	1/0	37	SMNS 19106
	CY2f	Cyprus	Karavas Alsavcak Bay, rocky shore and cave on western side of bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'13''N 33°13'06''E	0-1	23 May 1997	2/1	36-41	SMNS 19089
	CY2g	Cyprus	Karavas Alsavcak Bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'12''N 33°13'07''E	0-1	22 May 1997	2/0	38-42	SMNS 19089
	CY2h	Cyprus	Karavas Alsavcak Bay, small island on eastern side of bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'13''N 33°13'15''E	0-1	18 May 1997	2/0	34-37	SMNS 19054
	CY2i	Cyprus	Karavas Alsavcak Bay, small island on eastern side of bay, 9 km west of Kyrenia/Kyreneia/Girne	35°21'13''N 33°13'15''E	0-1	19 May 1997	4/1	37-44	SMNS 19059
	TK1	Turkey	Side, Pamphylia	36°45'58''N 31°23'04''E	n.a.	5 June 1988	1/0	43	SMNS 8402
	TK2a	Turkey	Kas, Lycia, Antalya Province	36°11'30''N 29°38'33''E	n.a.	9 June 1988	3/0	36-42	SMNS 8408
	TK2b	Turkey	Kas, southern harbour jetty, Lycia, Antalya Province	36°11'46''N 29°38'33''E	n.a.	7 June 1988	2/0	40-42	SMNS 8406
	TK2c	Turkey	Kas, Lycia, Antalya Province	36°11'30''N 29°38'33''E	n.a.	10 June 1988	2/0	42-45	SMNS 8407
	TK2d	Turkey	Kas, Lycia, Antalya Province	36°11'30''N 29°38'33''E	n.a.	11 June 1988	6/0	38-47	SMNS 8389
	TK3	Turkey	Torba, ca. 12 km north of Bodrum, Karia	37°07'24''N 27°23'47''E	n.a.	19 June 1988	7/0	35-50	SMNS 8373
	TK4a	Turkey	Bodrum, Karia, Egean Sea	37°01'53''N 27°25'38''E	n.a.	16 June 1988	5/1	39-58	SMNS 8392
	TK4b	Turkey	Bodrum, Karia, Egean Sea	37°01'53''N 27°25'38''E	n.a.	17 June 1988	8/2	35-50	SMNS 8390
	TK5	Turkey	Orag Island, Karia	36°58'35''N 27°35'39''E	n.a.	29 June 1988	6/0	35-52	SMNS 8375
	TK6	Turkey	Bay south of Ayvalik, Province Balikesir, Egean Sea	39°14'N 26°38'E	n.a.	3 June 1969	1/1	58	SMNS 13607
	TK7	Turkey	Erdek, west of Bandirma, Marmara Sea	40°24'N 27°48'E	n.a.	28 May 1969	10/-	40-59	SMNS 14326
	GR1a	Greece	Elounda, north of Aghios Nikolaos, Kreta/Crete Island	35°24'N 24°40'E	n.a.	12 Aug. 1971	4/-	42-50	SMNS 14371
	GR1b	Greece	Elounda, north of Aghios Nikolaos, Kreta/Crete Island	35°24'N 24°40'E	n.a.	10 Aug. 1971	3/-	42-50	SMNS 14369
	GR2	Greece	Cyclades Is. Kythnos Is.	36°43'35''N 25°16'35''E	0-2	24 Oct. 2004	2/2	42-55	IIPB
	GR3	Greece	Kyra Island, Gulf of Epidavros	37°37'30''N 23°12'00''E	n.a.	20 July 1970	7/-	28-36	SMNS 14361
	GR4	Greece	Aiyina Island, southern tip, Saronian Gulf	37°41'N 23°24'E	n.a.	20 Aug. 1969	1/-	47	SMNS 14366
	GR5	Greece	Porto Zografou, 24 km southeast of Nikiti, east coast, Sithonia, Chalkidiki	40°06'N 23°54'E	n.a.	18 Aug. 1994	2/-	59-62	SMNS 15737
	GR6	Greece	Palaiokeastrizza, Korfu/Corfu Island	39°43'N 19°38'E	n.a.	11 July 1977	2/-	57-64	SMNS 8395
	MO1	Montenegro	Bay north of Budva, right side of river mouth	42°16'30''N 18°50'30''E	n.a.	7 May 1977	1/-	55	SMNS 13609
	MO2	Montenegro	Bay of Kotor, at Bijela	42°27'N 18°41'E	n.a.	20 May 1969	1/-	45	SMNS 14327
	CR1	Croatia	Lokrum Island, west shore, Dubrovnik	42°37'36''N 18°07'07''E	n.a.	21 Sep. 1987	6/0	32-53	SMNS 8391
	CR2a	Croatia	Tatinica, northwest coast, Mljet Island	42°46'34''N 17°27'59''E	n.a.	18 Sep. 1987	1/0	46	SMNS 8400

TABLE 1 (cont.). – Specimens of the two *Tripterygion* species collected at different localities of the Mediterranean and Atlantic adjacent waters. The number of individuals used for morphological (Nm) and molecular (Ng) analyses, for each locality, are detailed.

Map code	Country	Locality	Latitude/ Longitude	Depth (m)	Collection date	Nm/ Ng	LT range (mm)	Catalogue number
CR2b	Croatia	southwest coast, Mljet Island	42°45'52''N 17°21'51''E	n.a.	17 Sep. 1987	3/0	54-68	SMNS 8404
CR3	Croatia	Sestrice Island, near Orebic, Peljesac	42°56'N 17°08'E	n.a.	9 Aug. 1963	2/-	62-63	SMNS 14325
CR4	Croatia	Orebic, Peljesac	42°56'N 17°08'E	n.a.	7 Aug. 1963	1/0	52	SMNS 14370
CR5	Croatia	Gojak Island, 12 km southeast of Kardeljevo	42°57'N 17°27'E	n.a.	20 Aug. 1963	5/0	42-47	SMNS 14362
CR6	Croatia	Badija Islet, beach at north coast of islet, east of Korčula city, Korčula Island	42°57'28''N 17°09'43''E	n.a.	9 Sep. 1987	4/0	41-51	SMNS 8399
CR7	Croatia	Podaca, 12 km northwest of Ploče	43°09'N 17°15'E	n.a.	6 Aug. 1963	1/0	51	SMNS 14368
CR8a	Croatia	Hvar City, Hvar Island	43°10'09''N 16°26'31''E	n.a.	26 Sep. 1987	6/0	39-54	SMNS 8401
CR8b	Croatia	Jerolim Islet, near Hvar,	43°09'28''N 16°23'31''E	n.a.	25 Sep. 1987	4/0	27-49	SMNS 8393
CR8c	Croatia	Lesina/Hvar City, Hvar Island	43°10'N 16°27'E	n.a.	cat. entry June 1854	2/0	53-57	SMNS 420
CR9	Croatia	Bay of Rogoznica, at Rogoznica	43°31'10''N 15°59'00''E	n.a.	8 Sep. 1987	1/0	22	SMNS 25179
CR10a	Croatia	Biograd	43°55'N 15°23'E	n.a.	7 Aug. 1959	2/0	38-45	SMNS 13365
CR10b	Croatia	Biograd	43°55'N 15°23'E	n.a.	4-6 Aug. 1961	5/0	38-57	SMNS 13364
CR11	Croatia	Karlobag, coast at northern entrance into town	44°32'N 15°04'E	n.a.	5 Aug. 1963	2/0	50-55	SMNS 13605
CR12	Croatia	Gavza Bay, 3 km northwest of Cres City, Cres Island	44°59'24''N 14°23'24''E	n.a.	2 May 1989	2/0	61-64	SMNS 8664
CR13a	Croatia	Osor, Cres Island	44°42'N 14°23'E	0.7	12 Sep. 1989	2/0	36-43	SMNS 9428
CR13b	Croatia	Osor, Cres Island	44°42'N 14°23'E	n.a.	Sep. 1989	3/0	35-61	SMNS 9425
CR13c	Croatia	Osor, Cres Island	44°42'N 14°23'E	0.2	27 Sep. 1989	3/0	32-55	SMNS 9423
CR13d	Croatia	Osor, Cres Island	44°42'N 14°23'E	n.a.	Sep. 1990	4/0	35-63	SMNS 11236
CR14	Croatia	Cres City, Cres Island	44°57'24''N 14°24'21''E	n.a.	3 May 1989	3/0	43-63	SMNS 9214
CR15a	Croatia	Zlatne Stijene, 5 km south of Pula, Istria	44°50'30''N 13°50'30''E	n.a.	10 June 1978	2/0	60-63 8410	SMNS
CR15b	Croatia	Zlatne Stijene, 5 km south of Pula, Istria	44°50'30''N 13°50'30''E	n.a.	9 June 1978	5/0	47-60	SMNS 8396
IT1	Italy	Lecce, Harbour	40°13'45''N 18°06'30''E	0-2	5 June 2004	5/4	36-59	IIPB
IT2	Italy	Sicily Is., Messina Harbour	38°11'N 15°33'E	0-2	4 Jan. 2006	5/5	49-63	IIPB
FR1	France	Corsica Is., Ile Rousse	42°37'39''N 8°55'37''E	0-2	24 Aug. 2004	2/2	28-31	MNCN
FR2	France	Nice, Harbour	43°25'16''N 7°08'24''E	0-2	13 Mar. 2005	2/2	49-58	MNCN
SP1	Spain	Port de la Selva, Harbour	42°42'38''N 3°19'50''E	0-2	12 Aug. 2004	12/1	43-63	IIPB
SP2	Spain	Blanes, St.Francisc Bay	41°40'09''N 2°48'15''E	0-2	30 Jul. 2002	9/2	45-65	MNCN
SP3	Spain	Tarragona, Altafulla	41°05'35''N 1°13'45''E	0-2	20 Jul. 2003	19/3	51-62	IIPB
SP4	Spain	Columbretes Is., La Foradada	39°53'50''N 0°41'15''E	0-2	4 Aug. 2002	4/3	38-47	MNCN
MA1a	Malta	Cirkewwa/Paradise Bay, southwest corner of bay, northwest coast, Malta Island	36°58'56''N 14°19'56''E	n.a.	10 Apr. 1974	1/-	58	SMNS 13045
MA1b	Malta	Cirkewwa/Paradise Bay, southwest corner of bay, northwest coast, Malta Island	36°58'56''N 14°19'56''E	0-1.5	10 Aug. 2005	7/-	30-44	SMNS 24888
MA1c	Malta	Cirkewwa/Paradise Bay, southwest corner of bay, northwest coast, Malta Island	36°58'56''N 14°19'56''E	0-1.2	12 Aug. 2005	4/-	29-47	SMNS 24911
MA1d	Malta	Cirkewwa/Paradise Bay, southwest corner of bay, northwest coast, Malta Island	36°58'56''N 14°19'56''E	0-1.5	11 Aug. 2005	1/-	25	SMNS 24899

TABLE 1 (cont.). – Specimens of the two *Tripterygion* species collected at different localities of the Mediterranean and Atlantic adjacent waters. The number of individuals used for morphological (Nm) and molecular (Ng) analyses, for each locality, are detailed.

	Map code	Country	Locality	Latitude/ Longitude	Depth (m)	Collection date	Nm/ Ng	LT range (mm)	Catalogue number
<i>Tripterygion tartessicum</i>	SP5	Spain	Menorca Is., Fornells Bay	40°04'23''N 4°08'31''E	0-2	5 Jul. 2002	2/2	50-60	IIPB
PARATYPES	SP6	Spain	Formentera Is., Punta Prima	38°44'N 1°25'14''E	0-2	7 May 2003	2/2	59-60	MNCN
PARATYPES	SP7	Spain	Cabo La Nao, Dènia - Les Rotes	38°51'N 0°07'E	0-2	5 May 2005	4/4	48-63	IIPB
PARATYPES	SP8	Spain	Cabo de Palos, Phare	37°37'57''N 0°41'56''W	0-2	30 Oct. 2002	12/2	41-60	IIPB
PARATYPES	SP9	Spain	Cabo de Gata, Aguamarga, Almería	36°59'43''N 1°53'41''W	0-2	26 Oct. 2002	16/1	38-67	MNCN
PARATYPES	SP10	Spain	Tarifa, Las Palomas Is.	36°00'15''N 5°36'30''W	0-2	20 Oct 2003	2/2	42-65	MNCN
PARATYPES	SP11	Spain (Africa)	Ceuta, Harbour	35°53'N 5°18'W	0-2	12 Feb. 2005	3/3	28-53	MNCN
HOLOTYPE	SP12	Spain	Cádiz, Puercas Phare	36°18'N 6°12'W	0-2	22 Oct. 2003	1/	67	IIPB 15/2005
PARATYPES	SP12	Spain	Cádiz, Puercas Phare	36°18'N 6°12'W	0-2	22 Oct. 2003	12/2	31-52	IIPB 15/2005
PARATYPES	SP13a	Spain	1 km SW of Punta de la Chullera, at Torreguadiaro (150 m northeast), Cádiz, Andalucía	36°18'23''N 5°15'39''W	0-1.5	14 Aug. 2004	2/0	72-73	SMNS 24307
PARATYPES	SP13b	Spain	1 km SW of Punta de la Chullera, at Torreguadiaro (150 m northeast), Province Cádiz, Andalucía	36°18'23''N 5°15'39''W	0-1.5	19 Aug. 2004	2/0	63-77	SMNS 24327
PARATYPES	MC	Morocco	Plage David	n.a.	n.a.	June 1985	11/-	42-69	SMNS 13516
PARATYPE	TU1a	Tunisia	Rocky cape, 4 km east of Tabarca, 66 km east of Bone/Annaba (Algeria)	36°57'33''N 8°47'54''E	0.1-2	2 June 1998	1/-	58	SMNS 20366
PARATYPE	TU1b	Tunisia	Rocky cape, 4 km east of Tabarca, 66 km east of Bone/Annaba (Algeria)	36°57'33''N 8°47'54''E	0-1.8	27 May 1998	1/-	46	SMNS 20356
PARATYPES	TU1c	Tunisia	Rocky cape, 4 km east of Tabarca, 66 km east of Bone/Annaba (Algeria)	36°57'33''N 8°47'54''E	0-0.6	23 May 1998	2/-	52-61	SMNS 20342

(0): no amplifications were done, (-): amplifications were done but they did not succeed, (n.a.): no available data. The holotype and paratypes are labelled; the catalogue number for each individual is shown. (IIPB): Instituto de Ciencias del Mar de Barcelona, (SMNS): Staatliches Museum fuer Naturkunde Stuttgart. The first two letters in the map code identify each country, the number identifies the locality and the lower case letter identifies different collection dates.

resulting in 15000 trees. The first 1500 trees were eliminated since they did not reach the stationarity of the likelihood values and the rest were used to construct the consensus tree and obtain the posterior probabilities of the branches.

SYSTEMATIC ACCOUNT

Tripterygion tartessicum n. sp. (Figs. 2 and 3a)

Etymology. The name *tartessicum* referred to the old Spanish culture (*Tartessos*, at least dating from 1000 BC) located on the south coast of the Iberian peninsula (in modern Andalusia, Spain), where the new species is partially distributed.

Morphological description. Body elongate and compressed, greatest height at base of anal fin, being

about one-sixth total length. Scales ctenoid, covering entire body except base of pectoral fin and ventral abdominal region back to vent. Lateral line having two sections: anterior section with 20 (19-22) pored scales, posterior section with 22 (21-24) notched scales, having 42 (40-46) in total. Upper, anterior, section commencing at upper angle of opercular opening, slightly curving up over pectoral fin base and running parallel to dorsal profile to point below last 1-3 rays of second dorsal fin; canal running across exposed width of each scale. Lower, posterior, section commencing below, and in front of last scale or two of upper section, running along the mid-line of tail to caudal fin base; each scale with shallow notch in free-edge tip.

Three dorsal fins with III + XVI + 13 (III + XVI + XVIII + 12-13) rays. First dorsal fin lower than second and second higher than third. First just above pre-operculum, rays being of equal height. Second separated by short interspaces, origin slightly behind

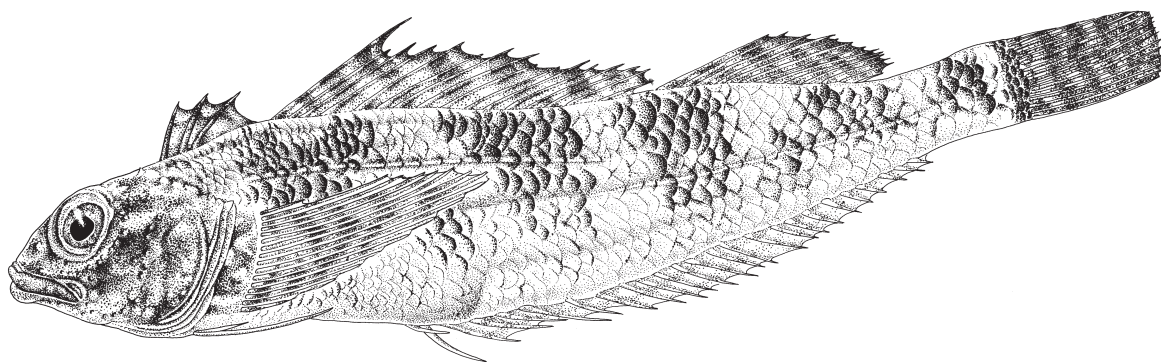


FIG. 2. – *Tripterygion tartessicum* Holotype, IIPB 15/2005, male, 67 mm TL, from SP12.

base of the pectoral fin; first ray longest, in mature males being nearly twice as long as rays in middle region, with distal half not united by membrane with following ray. Base of third fin about 0.6 length of second dorsal fin base.

Caudal fin truncate, with X (IX-X) principal branched rays, and 2 (2-3) procurrent lower and upper.

Anal fin elongate and of uniform height, with II + 23 (II + 22-24) rays. Anteriorly, 2 weak, slender, unsegmented rays, first shorter than second, which is slightly longer than the first segmented ray; succeeding rays united by membrane and decreasing in length posteriorly.

Pectorals long and broad, slightly overreaching mid-length of second dorsal fin and base of anal fin; with 16 (15-16) rays, upper three rays short and simple, remainder branched; ninth ray, counted from upper edge, longer than others.

Pelvic fins with one short spine and two slender and segmented rays; longest ray reaching mid-length of pectoral fin.

Head broad, scale less, profile acute, lips prominent. Head length 0.19 (0.16-0.22) times total length (TL). Orbit large, almost circular, diameter 0.32 (0.28-0.51) times head length, upper edge forming ridge along upper head profile. Pre-orbital and pre-dorsal lengths 0.05 and 0.14 times total length, respectively (0.06 and 0.18). Interorbital region concave. Mouth nearly horizontal, maxilla extending to level of front of pupil. Gill membrane continuous across throat. Teeth conical, in band in upper and lower jaws. Anterior nostril tubular, posterior nostril close to orbit edge. Cephalic canal pores as illustrated in Figure 3a, with preopercular-dentary series complete. The mandibular pore formula (Fricke, 1997) was 3+2+3 (3-4+2+3-4), basically depending on the fish TL, suggesting that an increase in length could be associated with the appearance of a new

pore in both dentaries. However, no significant relationship was found between this formula and TL, or between the two species. The interorbital series 2 (2-4) opened singly from the upper interorbital region to the upper lip. The preopercular series opened singly along the lower side of the preopercular canal, opening in pairs on the posterior pre-opercular edge. The nasal and suborbital canals usually opened in pairs, running along the lower and the posterior margins of the orbit; nasal pores 3 (1 to 3) placed in front of the anterior border of the eye;

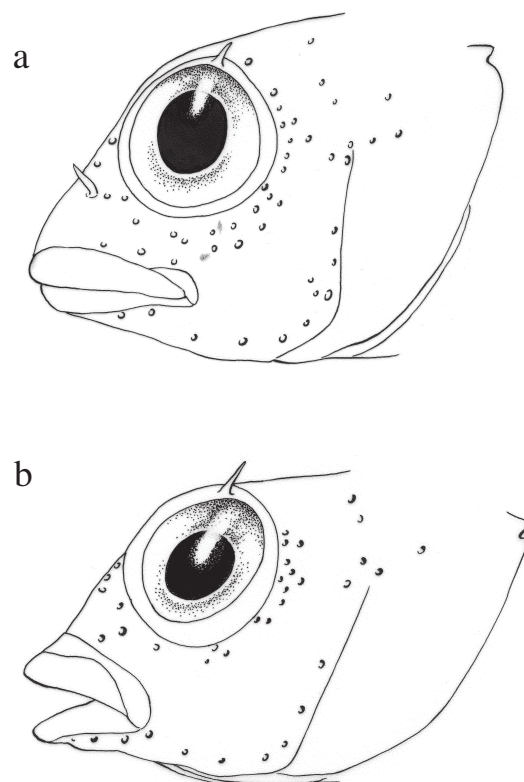


FIG. 3. – Variation in the cephalic pore system between *T. tartessicum* (a, female 55 mm from SP9) and *T. tripteronotus* (b, female 58 mm from SP1).

outer branch of suborbital pores ending as a cluster of pores in the postorbital region. Some nuchal pores running from the upper part of the operculum across the nape to the opposite side. Postocular canal with single pores (Fig. 3a).

Colouration in life. Mature males during reproduction period (March–August): black head, extending posteriorly to first dorsal, laterally to operculum edge, and ventrally including branchiostegal membranes across throat, base of pectoral fins and pelvic rays. Red body. Caudal fin with 4 red bars (dark brown in preserved specimens). First dorsal fin rays and membrane heavily pigmented. Anal fin with dusky marks, membrane hyaline. Pectoral fins hyaline, median rays with dusky margins on basal third. The rest of the year their colouration is as females or immature males (sneakers).

Females and immature males (sneakers): head and body light brown with dark bars across flanks, last bar not forming extension onto base of caudal fin. First dorsal fin heavily pigmented both on rays and membrane, second and third dorsal fins with brownish bars. Caudal fin with 4 distinct brownish bars.

Habitat. The new species inhabits similar habitats to *T. tripteronotus*: shallow rocky shores to 6 m, preferably between 0 and 3 m; in light-exposed and shady biotopes dominated by algal communities (e.g. *Corallina elongata*, *Cladophora* spp., *Litophyllum* spp., *Enteromorpha* spp.). Nests are usually situated in sciaphyl habitats dominated by steep rocky zones, without arborescent algae.

Comparison between *T. tripteronotus* and *T. tartessicum*

Background

Tripterygion tripteronotus was described by Risso (1810) as *Blennius tripteronotus*, from specimens collected in Nice (France; FR2). Unfortunately the types seem to be lost. Subsequently, the species was named as *T. nasus* (Risso, 1826) from material collected in Nice (France; FR2), *T. melaenocephalus* (Cocco, 1829) from specimens collected in Messina (Italy; IT2), and *Tripterygium nikolskii* (Maksimov, 1909) from the Crimea (Ukraine, Black Sea). These names were considered as junior synonyms of *T. tripteronotus* (see Hureau and Monod, 1973; Zander, 1986). Zander and Heymer (1970, 1976) mentioned

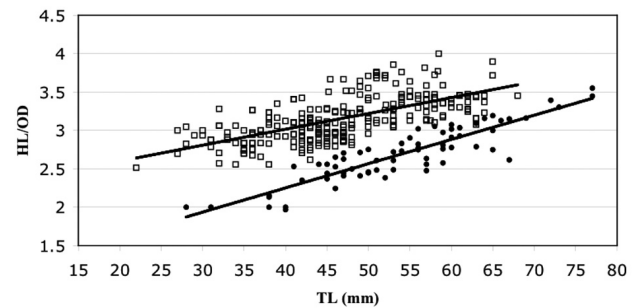


FIG. 4. – Plotted relationship between Total Length (TL, mm) and Head Length (HL, mm) / Orbital Diameter (OD, mm) for all measured individuals from the two species. (●): *T. tripteronotus*, (□): *T. tartessicum*. Regression equations are $HL/OD = 0.0207TL + 2.1884$ ($R^2 = 0.42$) and $HL/OD = 0.0315TL + 0.991$ ($R^2 = 0.80$) respectively.

some slight morphological differences between specimens from different localities (NW Mediterranean and Mediterranean coasts of Morocco and Israel), although they were considered as intraspecific variations.

Morphological data

The morphological comparison of the present material of *T. tartessicum* with specimens of *T. tripteronotus* from different localities of the Mediterranean and adjacent waters showed that there are only slight differences between the two species. They can be differentiated by a morphometric measurement: the orbital diameter (OD) is significantly longer in the new species (mean ratio head length/orbital diameter = 2.69 ± 0.36) than in *T. tripteronotus* (3.16 ± 0.29 ; Mann-Whitney U-test, $p < 0.05$). When HL/OD was represented in front of TL, two well-differentiated and almost non-overlapping groups were found, corresponding to both species (Fig. 4). In order to assure this differentiation, a multivariate analysis of covariance (MANCOVA) was implemented using TL as the covariate and HL/OD as the dependent variable. The results showed a highly significant differentiation between the two groups ($F = 415.72$, $p < 0.001$).

The first ray of the second dorsal fin of the mature males has the distal half not united by a membrane with the following ray in *T. tripteronotus*, whereas the first two rays can be united by a membrane from their respective tips in *T. tartessicum*. Additionally, the caudal fin usually has four red or brownish bars (black in preserved specimens) in the new species, whereas these bars are usually not distinct in *T. tripteronotus*. These two differences are similar to the ones described by Zander and Heymer

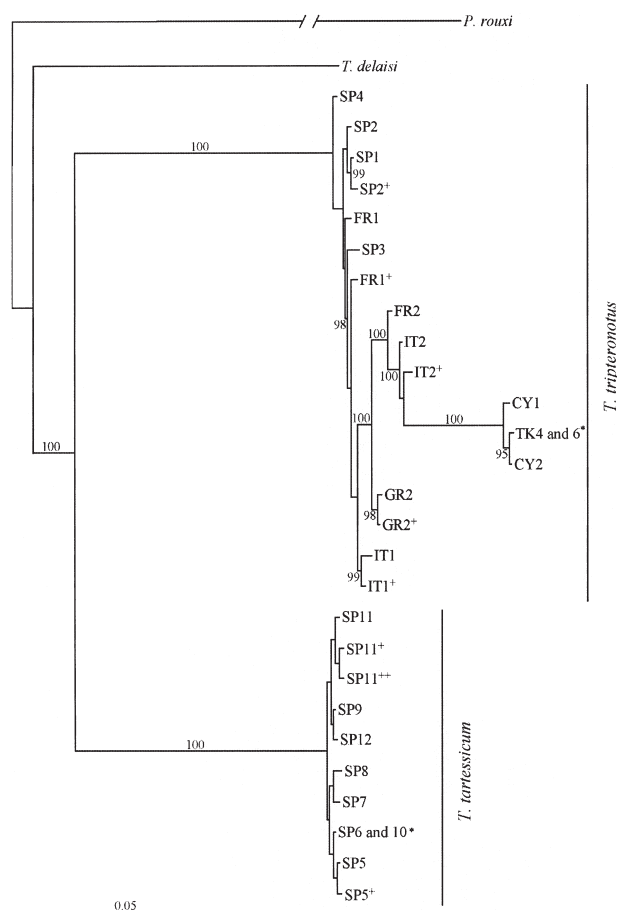


FIG. 5. – Haplotype tree inferred from Bayesian Inference for *T. tartessicum* and *T. tripteronotus* species using all genes together. Only probabilities above 95% are shown; (+ and ++): different haplotypes found in the same locality; (*): the same haplotype found in different localities. See Table 1 for locality abbreviations and further details or Figure 1 for a quick geographical location.

(1976), although they should be considered with caution since they were not always observable in all the individuals collected.

We also observed that the mating season seems to start slightly later in the new species. In fact, all mature males of *T. tripteronotus* are active on the Catalan coast (NE Spain) in early May, whereas most mature males of the new species are not active at this time on the coasts of Murcia and Almeria (SE Spain).

Molecular data

We analysed a total of 1732 bp for all genes combined in 55 individuals (18 *T. tartessicum* and 37 *T. tripteronotus*). A total of 10 haplotypes were found for *T. tartessicum*, whereas 17 were shown for *T. tripteronotus* (Fig. 5). Generally, all individuals from one locality shared the same haplotype, and

TK4-TK6 and SP6-SP10 also shared the same haplotype. However, at some localities (SP2, FR1, IT1, IT2, GR2, SP5 and SP11) more than one haplotype was found. For each of the four mitochondrial genes the sequence obtained was of 419 bp for 12S rRNA, 699 bp for 16S rRNA, 73 bp for tRNA-valine and 541 bp for Cytochrome Oxidase I. All genes used showed a similar percentage of parsimony informative sites (chi-square = 7.57 $p = 0.36$) ranging from 2.74 to 10.35%, but only the RNA genes had similar variable sites (chi-square = 7.52 $p = 0.18$) ranging from 10.50 to 14.59%, the percentage being higher for COI (19.41%). For the COI protein coding gene, third codon positions were 54.19% variable, second codon positions were invariant and first codon positions were 4.47% variable. The Ts/Tv ratio ranged between 2.61 (16S) and 6.00 (COI) with 4.13 for 12S, and 2.63 for tRNA-valine. There was no evidence of sequence saturation in the analysed genes. For each gene sequence the goodness-of-fit test showed homogeneous base composition across taxa ($P = 1.00$) and the partition homogeneity test showed no significant heterogeneity between genes (P_{ILD} range from 0.15 to 1.00), and although there is no generally accepted p-value for significant results, most authors agree to combine data when p-values are greater than 0.05 (Cristescu and Hebert, 2002; Russello and Amato, 2004).

As assessed in Carreras-Carbonell *et al.* (2005), two well-supported clades for *T. tripteronotus* (northern and southern) were found with posterior probabilities of 100%. The southern clade belonging to *T. tartessicum* showed no well-supported structure pattern between different localities. However, the northern clade (*T. tripteronotus*) showed several well-supported subclades that could be related to defined geographical areas (e.g. Cyprus and Turkey), indicating some degree of isolation between different populations (Fig. 5).

Molecular divergence between *T. tripteronotus* and *T. tartessicum* ranges between 9.14% (COI) and 2.79% (tRNA-valine), with a mean value combining all genes of 6.89% (Table 2). No genetically and/or morphologically hybrid populations or individuals were found.

DISCUSSION

The new species is geographically distributed along the southern coast of Spain, from Cape La Nao

TABLE 2. – Polymorphism and divergence within and between species, for each gene separately and all genes together (mean $\pm$ SD percentage).

	COI	12S	16S	tRNA-valine	All genes
<i>T. tripteronotus</i>	1.37 $\pm$ 0.40	1.24 $\pm$ 0.30	1.86 $\pm$ 0.42	0	1.33 $\pm$ 0.03
<i>T. tartessicum</i>	0.25 $\pm$ 0.08	0.24 $\pm$ 0.12	0.29 $\pm$ 0.05	0	0.15 $\pm$ 0.02
<i>T. tripteronotus</i> vs. <i>T. tartessicum</i>	9.14 $\pm$ 2.01	5.32 $\pm$ 1.78	6.72 $\pm$ 0.95	2.79 $\pm$ 0	6.89 $\pm$ 0.73
<i>T. tripteronotus</i> vs. <i>T. delaisi</i>	13.58 $\pm$ 3.92	8.23 $\pm$ 3.09	10.85 $\pm$ 2.81	11.84 $\pm$ 0	11.03 $\pm$ 2.60
<i>T. tartessicum</i> vs. <i>T. delaisi</i>	14.80 $\pm$ 6.98	6.87 $\pm$ 3.44	8.22 $\pm$ 2.88	8.70 $\pm$ 0	9.90 $\pm$ 2.97
<i>T. tripteronotus</i> vs. <i>P. rouxi</i>	16.08 $\pm$ 4.64	24.97 $\pm$ 9.31	26.25 $\pm$ 7.25	18.56 $\pm$ 0	22.61 $\pm$ 5.32
<i>T. tartessicum</i> vs. <i>P. rouxi</i>	18.49 $\pm$ 8.72	24.64 $\pm$ 12.32	24.59 $\pm$ 8.61	16.44 $\pm$ 0	22.35 $\pm$ 6.71

(SP7) to the Gulf of Cadiz (SP12), the Balearic Islands (SP5 and SP6), and northern Africa, from Plage David (MC; Morocco, Atlantic Ocean) to Tunisia (TU1) (see Fig. 1). The eastern boundary in the distribution of the new species is unfortunately unknown. Some morphological characteristics (e.g. rays of the second dorsal fin and caudal bands) of the specimens collected in Israel by Zander and Heymer (1970, 1976) are closely related to those observed in the new species, suggesting the presence of *T. tartessicum* in that area. However, as we have mentioned above, these morphological characters are not constant, and unfortunately we could not analyse specimens from this locality. Future studies are recommended to confirm the taxonomic position of this material.

T. tripteronotus is restricted to the northern Mediterranean basin, including the NE coast of Spain (from SP4 to SP1), France (FR2 and also Corsica Is., FR1), Italy (IT1 and also Sicily Is., IT2), the Adriatic Sea (CR1-15 and MO1-2), Malta Is. (MA1), the Aegean Sea, including the coasts of Greece (GR3-5) and Turkey (TK3-6), as well as the Cyclades Islands (GR2) and Crete (GR1), the Marmara Sea (TK7), the Mediterranean Turkish coast and Cyprus (TK1-2 and CY1-2) (see Fig. 1).

The individuals from Nice (FR2) and Messina (IT2) were grouped within the northern clade of *T. tripteronotus*, suggesting that all specimens from these localities belonged to the species described by Risso (1810). Therefore, *T. melaenocephalus*, described by Cocco (1829), can be considered as a junior synonym of *T. tripteronotus*, in agreement with previous studies (e.g. Zander, 1986). The specimens from the Black Sea, originally identified as *T. nikolskii* (Maksimov, 1909) and synonymised with *T. tripteronotus*, could not be analysed. However, the presence of *T. tripteronotus* on the Aegean coasts of Greece and Turkey, as well as in the Marmara Sea, suggests that the specimens from the Black Sea may belong to *T. tripteronotus* or *T. nikolskii*, but not to the new species.

Our results confirm the validity of subtle morphological characters for distinguishing species of the genus *Tripterygion*, and the existence of a cryptic species, as occurs in other fish taxa (Gleeson *et al.*, 1999; Henriques *et al.*, 2002; Yamazaki *et al.*, 2003). Nevertheless, the criteria used to designate distinct species based on molecular data are always controversial (Cracraft, 1989; Avise, 1994). The genetic divergence between *T. tripteronotus* and *T. tartessicum* is 9.14% for COI, 5.32% for 12S and 6.72% for 16S, similar to the divergence observed between other fish taxa. Yamazaki *et al.* (2003), using COI, found a sequence difference of 9.10 $\pm$ 0.36% between two cryptic species of brook lamprey. For 16S, genetic distances between congeneric species of the families Soleidae, Mullidae and Apogonidae range between 4.6 and 11.70% (Tinti *et al.*, 2000; Apostolidis *et al.*, 2001; Mabuchi *et al.*, 2003). Finally, for 12S the mean genetic distance between congeneric species of the genus *Coryphaenoides* was 3.31% (Morita, 1999), 4% within the genus *Macullochella* (Jerry *et al.*, 2001) and a mean of 6.5% within different blenniidae genera (Stepien *et al.*, 1997). Henriques *et al.* (2002), in a revision of the genus *Lepadogaster* (Teleostei: Gobiesocidae), observed that the minimum distance between valid species was 3% at 12S rRNA. Furthermore, Almada *et al.* (2005b), using 12S and 16S genes, showed that the genetic differences between clearly morphologically differentiated European blennioid species of the genus *Parablennius* and *Lipophrys* were even smaller (1.3-1.6%). Within the genus *Tripterygion*, *T. tripteronotus* and *T. tartessicum* showed the smallest divergence, indicating a more recent speciation event (Carreras-Carbonell *et al.*, 2005).

The estimated divergence time found between the two species was approximately 3.17 Myr when the evolutionary rates of 0.81 $\pm$ 0.23%/Myr for 12S and 1.10 $\pm$ 0.23%/Myr for 16S inferred for the genus *Tripterygion* (Carreras-Carbonell *et al.*, 2005) were applied. This divergence could be caused by the

marine regressions during the Pliocene glaciations (2.7-3.6 Mya), when the sea level fell several meters. During the glaciations, a barrier could be formed between Cape La Nao (SP7) and the Balearic Islands (SP5 and SP6), acting as a separation between the two basins and allowing diversification between the two clades. However, we cannot discard the existence of a barrier elsewhere (e.g. the Gibraltar Strait) and a later expansion, the boundaries being the results of secondary contacts. Today, the low larval and adult dispersal capabilities of *Tripterygion* species (Heymer, 1977; Wirtz, 1978; Sabatés *et al.*, 2003; Carreras-Carbonell *et al.*, 2006) and the circulation regime that separates the northern from the southern basins (Send *et al.*, 1999) could be maintaining the distribution areas of the two species non-overlapping.

Key to the Mediterranean tripterygiids

Modified from Zander (1986).

- 1a. Profile of head acute with an arch of about 60°; lips protruding; head mask of territorial males extending to breast; females and non-territorial males with marbled head; body permanently red. *Tripterygion melanurus*
- 1b. Profile of head more obtuse with an arch of about 70°; lips not protruding; head mask not extending to breast; females and non-territorial males without marbled head 2
- 2a. Last dark bar of body forms a distinct black spot on caudal peduncle with an extension onto base of caudal finrays; body of territorial males yellow, head mask not extending to tip of pectoral fins 3
- 2b. Last dark bar of body not forming an extension onto base of caudal fin; body of territorial males red; head mask extending to tip of pectoral fins. 4
- 3a. During the courtship males draw a figure-of-8 swimming upwards into the water. Current distribution: Macaronesia *Tripterygion delaisi delaisi*
- 3b. During the courtship males draw a figure-of-8 swimming only on the bottom. Current distribution: Mediterranean Sea and Atlantic European coasts *Tripterygion delaisi xanthosoma*
- 4a. Eyes large, head length less than 2.5 times orbit diameter (in individuals between 2 and 5 cm) (Fig. 4) *Tripterygion tartessicum*
- 4b. Eyes moderately large, head length more than 2.5 times orbit diameter (in individuals between 2 and 5 cm) (Fig. 4) *Tripterygion tripteronotus*

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