Chondrostoma almacai, a new cyprinid species from the southwest of Portugal, Iberian Peninsula

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A b s t r a c t . A new species of Iberian cyprinid fish, *Chondrostoma almacai*, from the southwest of Portugal is described. Distribution area includes the Mira, Arade and Bensafrim drainages, previously considered to be inhabited by another phylogenetically related taxon, *Chondrostoma lusitanicum*. *Chondrostoma almacai* sp. nov. and *C. lusitanicum* present important morphological similarities, though significant differences were found for meristic and morphometric characters. The main diagnostic characters for *C. almacai* are the higher number of scales on the lateral line and above the lateral line, the lower number of right pharyngeal teeth, the longer snout and the smaller head depth. Furthermore, the new species has a small number of gill rakers and a larger predorsal distance, pelvic fin length and eye diameter. Previous molecular data, both mtDNA and nuclear markers (allozymes), suggested that *C. almacai* and *C. lusitanicum* are strongly differentiated sister-species. The morphological variability and geographic distribution of *C. lusitanicum* are reviewed and the most southerly populations from the southwest of Portugal are now recognized as the new species, *C. almacai*. It is recommended that both species be considered Critically Endangered according to the IUCN Red List Categories and Criteria [B1ab(ii,iii,iv)c(iv)+2ab(ii,iii,iv)c(iv) for *C. almacai* and A2ce+3ce+4ce for *C. lusitanicum*].

Key words: *Chondrostoma lusitanicum,* Cyprinidae, endangered, endemic, morphological markers

Introduction

The genus *Chondrostoma* Agassiz, a member of the Leuciscinae subfamily (Teleostei, Cyprinidae), is widely distributed all over Europe. Although being represented in central Europe by a reduced number of species with wide distributions, it presents several endemics in the northern Mediterranean drainages, from the Atlantic to the Caspian Sea, characterized by considerably restricted distribution areas. The molecular phylogeography of *Chondrostoma* was recently addressed by D u r a n d et al. (2003) and D o a d r i o & C a r m o n a (2004), but there is no consensus about the evolutionary processes and associated genetic divergence times. D u r a n d et al. (2003) concluded that a dispersalist model explains the evolution of the *Chondrostoma* genus, and considered two main radiation events: first, a Messinian dispersion around the Mediterranean during the Lago Mare phase (5.3-5.6 million years, MY) and second, during Upper Pliocene and Lower Pleistocene (1.0-2.2 MY), a Mesopotamian dispersion through the Black Sea to the Danube system. On the contrary, D o a d r i o & C a r m o n a (2004) support an evolutionary model based on vicariant events associated with the differentiation of the drainage systems: separation of the main lineages took place in the Middle-Upper Miocene (around 11.0 MY ago) and most species radiation occurred during the Pliocene (1.8-5.3 MY) with the formation of the current drainage systems.

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In the Iberian Peninsula, *Chondrostoma* is one of the four native cyprinid genera, along with *Anaecypris* Collares-Pereira, *Barbus* Cuvier, and *Squalius* Bonaparte, with a total of 11 endemic species according to D o a d r i o & C a r m o n a (2004): *Chondrostoma arcasii* (Steindachner, 1866); *Chondrostoma arrigonis* (Steindachner, 1866); *Chondrostoma duriense* Coelho, 1985; *Chondrostoma lemmingii* (Steindachner, 1866); *Chondrostoma lusitanicum* Collares-Pereira, 1980; *Chondrostoma macrolepidotum* (Steindachner, 1866); *Chondrostoma miegii* Steindachner, 1866; *Chondrostoma oretanum* Doadrio & Carmona, 2003; *Chondrostoma polylepis* Steindachner, 1864; *Chondrostoma turiense* Elvira, 1987; *Chondrostoma willkommii* Steindachner, 1866.

Chondrostoma lusitanicum is only found in the most southwestern part of the Iberian Peninsula, in Portuguese inland waters (Fig. 1). It was described by C o l l a r e s - P e r e i r a (1980) based on morphological grounds, as an arched-mouth *Chondrostoma* inhabiting the southern drainages of Portugal. However, some variability at the population level, mainly regarding the number of scales and the body shape, was subsequently reported (C o l l a r e s - P e r e i r a 1983) to exist between the most southern drainages (Mira and Arade) and those of the type-locality (Sado) and Tejo drainages further north. More recently, R o d r i g u e s (1993) confirmed the morphological differentiation of Mira specimens from the fish inhabiting the Samarra drainage (north of the Tejo), as well as the existence of a marked allozyme differentiation between the northern and the southern populations. In fact, molecular studies based on allozymes (C o e l h o et al. 1997b) and later on mitochondrial DNA data (M e s q u i t a et al. 2001), showed high levels of genetic differentiation for the extreme southwestern populations of *C. lusitanicum*. C o e l h o et al. (1998) already formally described two new species of *Squalius* (formerly considered as *Leuciscus*) in the extreme southwestern Portuguese rivers, *Squalius torgalensis* and *Squalius aradensis*. Although the description of a new *Chondrostoma* species has been also strongly suggested by genetic data (C o e l h o et al. 1997b, M e s q u i t a et al. 2001), its formal description is now presented under the designation of *Chondrostoma almacai* sp. nov. Since the populations of the new species were previously included within the distribution area of *C. lusitanicum* (Fig. 1), the morphological characterization and geographic distribution of this latter species are now reviewed.

Materials and Methods

The examined material was collected by seine nets and electrofishing and killed by lethal dose of anaesthetic, in accordance to the recommended ethics guidelines (A S A B 1998). It was deposited at the Museu Nacional de História Natural (Museu Bocage, MB), Lisbon, Portugal.

Collection of meristic and morphometric data follows methods of C o l l a r e s - P e r e i r a (1983), except otherwise noted. Measurements were taken using an electronic digital caliper and taken to the nearest 0.01 mm. The following abbreviations were used in the text and tables for the meristic and morphometric characters: lateral line scales, LLS; scales above lateral line, LLSA; scales below lateral line, LLSB; branched dorsal fin rays, DR; branched anal fin rays, AR; total gill rakers, GR; left pharyngeal teeth, LPT; right pharyngeal teeth, RPT; standard length, L_S [straight line distance from the anteriormost point of the snout (upper lip) to the base of the caudal fin (basis of central rays); H o w e s 2002]; head length, HL; head depth, HD; snout length (= preorbital length), SNL; eye diameter, ED; predorsal distance, PD; preanal distance, PA; predorsal depth, PDD; preanal depth, PAD; caudal peduncle depth (= minimum body depth), CPD; pelvic fin length, PF.
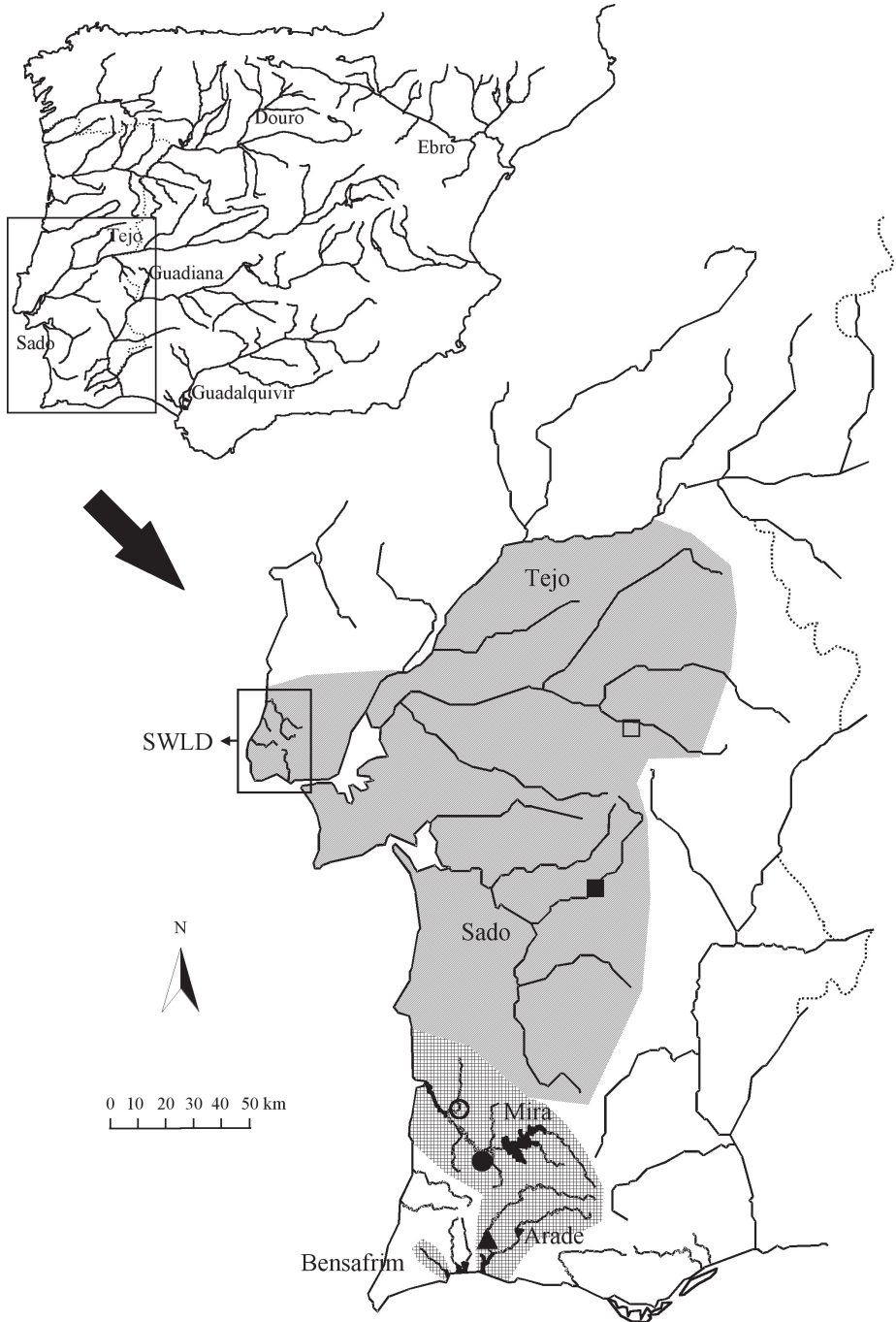
Fig. 1. *Distribution* range of *C. almacai* sp. nov. (Mira, Arade and Bensafrim drainages; ▨) and *C. lusitanicum* [most western tributaries of the south bank of Tejo drainage, Sado drainage and the small western littoral drainages (SWLD) of Lizandro, Samarra, Colares and Ossos; ▦] in the southwest of Portugal. Sampling site locations: Mira drainage, Defesa river (●) and Torgal river (○); Arade drainage, Odelouca river (▲); Sado drainage, Xarrama river (■); Tejo drainage, Freixo river (□).

To test if meristic and morphometric values were significantly different between samples of *C. almacai* sp. nov. (Arade and Mira drainages) and samples of *C. lusitanicum* (Sado and Tejo drainages), non-parametric Mann-Whitney *U*-tests were done on the meristics, on L_S and on the relative morphometric ratios (% of L_S or % of HL) using SPSS for Windows v.10.0.1 (SPSS Inc., Chicago).

In order to explore the multivariate data set, as a complement to the non-parametric Mann-Whitney *U*-test, and to identify the variables that contribute the most to the differences between the two species of *Chondrostoma*, principal component analyses (PCA) of meristic and morphometric data were performed for all the specimens analysed using the same software package, SPSS for Windows v.10.0.1 (SPSS Inc., Chicago). In order to study the size-free shape differences between species, the morphometric data were also analysed through Burnaby's size correction method (Burnaby Principal Component Analysis, BPCA; B u r n a b y 1966, R o h l f & B o o k s t e i n 1987) using the programme BURNABY PCA (available from N. MacLeod, Natural History Museum, London, http://www.nhm.ac.uk/ hosted_sites/paleonet/ftp/ftp.html). In the BPCA approach size and shape components were separated and a multivariate analysis of shape was accomplished eliminating the contribution of size on the second and following principal components (shape components) and restricting the size component to PC I (PC, principal component). This procedure is considered to be the most effective traditional morphometrics method for isolating shape from size variation (B o o k s t e i n et al. 1985, R o h l f & B o o k s t e i n 1987, P a r s o n s et al. 2003), to successfully differentiate morphologically similar populations and species of fish (e.g. B r o w n et al. 1992, J o n e s et al. 2002, P a r s o n s et al. 2003).

Morphometric and meristic data were analysed separately, but to accomplish a more powerful discrimination between forms, the first Burnaby adjusted principal component (BPC II) was plotted against the PC I of meristic data (H u m p h r i e s et al. 1981).

In the diagnosis and description, the extremes of variation found for the meristic characters are presented in round brackets and the holotype data in square brackets.

Results

Results from the meristic and morphometric analyses for all specimens examined, *C. almacai* sp. nov. (n = 71) and *C. lusitanicum* (n = 54), are presented in Table 1.

For the total samples, the Mann-Whitney *U*-test for L_S indicated highly significant differences ($P < 0.001$) between the two species. In accordance, the Mann-Whitney *U*-tests were also performed on subsamples of similar length classes and non-significantly different standard lengths ($P > 0.05$): *C. almacai* sp. nov., mean L_S = 71.97±10.61 mm (range 42.00–91.11 mm), n = 52; *C. lusitanicum*, mean L_S = 69.14±11.42 mm (range 44.38–93.16 mm), n = 49. The Mann-Whitney *U*-tests, comparing the subsamples from the distinct drainages, resulted in highly significant differences ($P < 0.001$) between the two groups for the meristic characters LLS, LLSA and RPT, and significant differences ($P < 0.005$) for GR. Concerning the morphometric characters, HD and PD (as % of L_S), and SNL (as % of HL) showed highly significant different values ($P < 0.001$) between samples of *C. almacai* sp. nov. and those of *C. lusitanicum*. The species were also significantly different ($P < 0.05$) for PF (as % of L_S) and for HD and ED (as % of HL).

Multivariate analysis for meristic characters showed that PC I accounts for 21.9% of the variation and PC II for 18.1%, and that the most significant weightings on PC I were from

Table 1. Meristic and morphometric data for all *C. almacai* sp. nov. (Mira and Arade drainages) and *C. lusitanicum* (Sado and Tejo drainages).

Character	*C. almacai* sp. nov. (n = 71)		*C. lusitanicum* (n = 54)	
	Mean±SD	Range	Mean±SD	Range
DR*	7.03±0.17	7.00–8.00	7.00±0.19	6.00–8.00
AR*	7.08±0.28	7.00–8.00	7.15±0.40	6.00–8.00
LLS*	49.34±1.94	45.00–53.00	47.25±1.60	43.00–51.00
LLSA*	11.05±0.39	10.50–12.00	10.41±0.41	9.50–11.00
LLSB*	4.16±0.39	3.50–5.50	4.15±0.37	3.50–5.00
GR*	31.13±1.85	25.00–35.00	32.30±3.20	27.00–41.00
LPT*	5.96±0.20	5.00–6.00	6.00±0.00	6.00–6.00
RPT*	4.99±0.12	4.00–5.00	5.35±0.48	5.00–6.00
L_S (mm)*	76.20±11.64	42.00–99.73	67.22±12.30	44.38–93.16
HL*†	22.12±1.18	17.15–24.80	22.64±0.01	20.54–25.28
HD*†	16.01±0.76	14.78–18.19	16.98±0.01	15.90–19.59
PD*†	52.68±1.10	49.70–54.95	51.99±0.01	49.35–56.28
PA*†	69.50±1.31	66.67–72.57	69.30±0.02	65.27–73.66
PDD*†	24.34±1.62	21.15–27.35	24.82±0.02	20.95–27.51
PAD*†	18.01±1.12	15.50–21.23	17.92±0.01	14.77–19.33
CPD*†	10.84±0.64	9.38–12.47	10.94±0.01	09.73–12.13
PF*†	15.86±1.13	13.32–18.14	15.41±0.01	13.47–17.79
HD*‡	72.53±4.36	65.31–90.07	75.11±0.04	67.85–81.82
SNL*‡	24.97±2.03	20.33–32.41	22.57±0.02	15.09–26.73
ED*‡	26.28±2.54	21.01–32.63	26.05±0.03	21.45–33.68

*, see Materials and Methods for abbreviations; †, % of L_S; ‡, % of HL.

LLSA, LLS and RPT, and on PC II from DR and AR (Table 2). Considering the PCA results for the morphometric data, PC I, PC II and PC III explain, respectively, 91.6%, 2.7% and 1.8% of the variation (Table 3). Moreover, all the characters weights on the first principal component presented the same sign and were of similar magnitude (Table 3), indicating that this axis represents general size-related variation (J o l i c o e u r & M o s i m a n n 1960,

Table 2. Relative eigenvalues, percentage of explained variance and weights of meristic data on the first three principal components for total samples of *C. almacai* sp. nov. and *C. lusitanicum*. Most significant weights on PC I and PC II in bold.

Meristics	PC I	PC II	PC III
Eigenvalue (% of variance)	1.752 (21.9)	1.448 (18.1)	1.164 (14.5)
DR*	0.079	**0.665**	-0.280
AR*	-0.306	**0.647**	-0.060
LLS*	**0.652**	0.341	0.451
LLSA*	**0.787**	0.204	-0.111
LLSB*	0.387	0.303	-0.427
GR*	-0.434	0.406	0.460
LPT*	-0.132	0.314	0.513
RPT*	**-0.502**	0.272	-0.457

* see Materials and Methods for abbreviations.

Table 3. Relative eigenvalues, percentage of explained variance and weights of morphometric data on the first three principal components for total samples of *C. almacai* sp. nov. and *C. lusitanicum*. Most significant weights on BPC II and BPC III in bold.

Morphometrics	PC I	PC II	PC III
Eigenvalue (% of variance)	10.079 (91.6)	0.302 (2.7)	0.200 (1.8)
	PC I	BPC II	BPC III
L_S*	0.304	0.000	0.001
HL*	0.291	-0.006	0.126
HD*	0.272	**-0.015**	-0.113
PD*	0.297	0.005	0.060
PA*	0.297	0.002	0.064
PDD*	0.313	-0.008	-0.438
PAD*	0.336	-0.004	-0.340
CPD*	0.310	-0.005	-0.280
PF*	0.321	0.006	0.085
SNL*	0.360	**0.018**	**0.744**
ED*	0.182	**0.011**	0.130

*, see Materials and Methods for abbreviations.

H u m p h r i e s et al. 1981, B o o k s t e i n et al. 1985), as corroborated by plotting PC I for morphometrics against L_S (Fig. 2c). Therefore, in order to study the size-free shape differences between species, the morphometric data were also analysed through BPCA, revealing that the size-independent shape components BPC II and BPC III were mainly defined, respectively, by SNL, HD and ED, and by SNL (Table 3), and that plotting PC I on L_S showed that this component, besides the high correlation with general size, presented a lack of shape-related species differentiation (Fig. 2c). *C. almacai* sp. nov. and *C. lusitanicum* were relatively separated when plotting all the individual scores on PC I and PC II for meristic characters, but less separated when BPC II and BPC III for morphometrics were used (Fig. 2a and b). More significant discrimination between the two species was obtained when morphometric and meristic results were complemented, and the Burnaby adjusted PC II was plotted against the PC I of meristic data (Fig. 2d). Although some overlap was observed, most differentiation occurred along PC I for meristics. Moreover, when comparing the non-parametric Mann-Whitney *U*-tests with the results of the multivariate analyses, the highly significantly different characters ($P < 0.001$) were totally in accordance with the most significant weightings of PC I for meristic, but not with the most significant weightings of BPC II for morphometrics. This indicates that the most discriminating characters between the two species were the number of scales above and on the lateral line, the number of right pharyngeal teeth, the snout length and the head depth.

Chondrostoma almacai sp. nov. [Fig. 3(a) and Table 1]
Holotype. MB 05-1870/21 October 2003, Torgal R., Odemira/Castelão (37°37'53" N; 8°37'32" W), Mira drainage, southwest Portugal; L_S 79.9 mm.

Paratypes. 21 specimens, MB 05-1870/1-20;22 October 2003, Torgal R., Odemira/ Castelão (37°37'53" N; 8°37'32" W), Mira drainage, southwest Portugal.

Non-type material. 31 specimens, MB 05-592a August 1986, Defesa R., Sabóia (37°29'57" N; 8°29'32" W), Mira drainage, southwest Portugal; 18 specimens, MB 05-1869
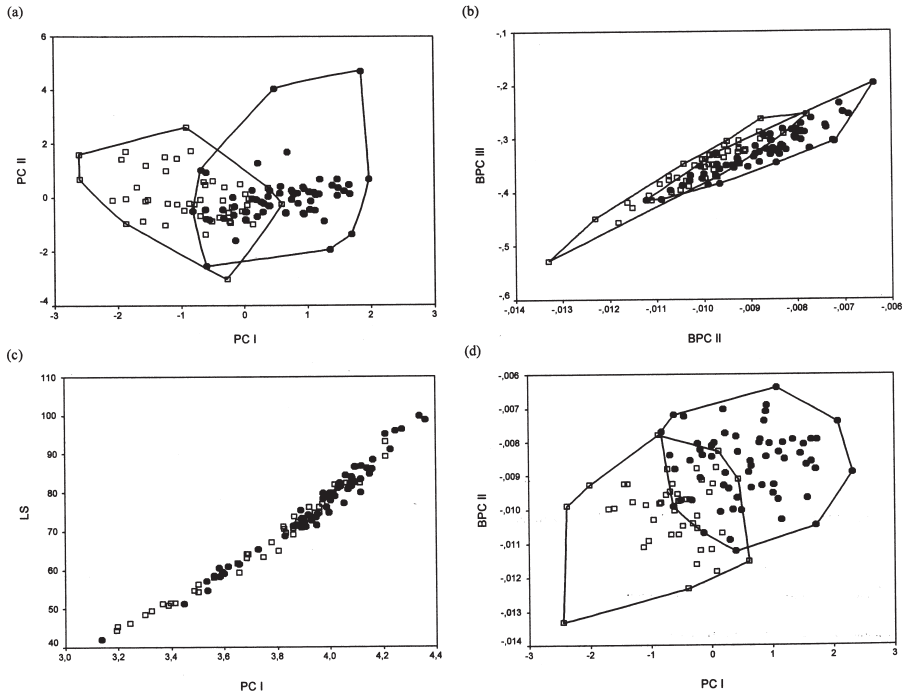
Fig. 2. Multivariate differentiation of all samples of *C. almacai* sp. nov. (●) and *C. lusitanicum* (□) according to: (a) PC I and PC II for meristics; (b) BPC II and BPC III for morphometrics; (c) PC I and L_S for morphometrics; (d) PC I for meristics and to BPC II for morphometrics.

October 1978, Odelouca R., Quinta de Santo Antão (37°12'29'' N; 8°30'36'' W), Arade drainage, southwest Portugal.

Diagnosis (Table 4). LLS (45)47–51(53) [51]; LLSA 10.5–11.5(12) [11]; RPT (4)5 [5]. In comparison to *C. lusitanicum*, *Chondrostoma almacai* sp. nov. has a higher mean number of scales on the lateral line and above the lateral line, a smaller number of right pharyngeal teeth, a smaller head but a larger snout and eye. Existence of one allozyme diagnostic locus, *PGDH*, with the presence of two exclusive and fixed alleles for *Chondrostoma almacai* sp. nov. (C o e l h o et al. 1997b).

Description. DR III 7(8) [7]; AR III 7(8) [7]; LLS (45)47–51(53) [51]; LLSA 10.5–11.5(12) [11]; LLSB 3.5–4.5(5.5) [4.5]; GR (25)29–33(35) [30]; LPT (5)6 [6]; RPT (4)5 [5]. Maximum total length of 148 mm according to data from C o l l a r e s - P e r e i r a (1983), with narrow body laterally compressed and rounded abdomen. Relatively small head, with length 17.15–24.80% in standard length; head depth about 1.5 times greater than CPD. Relatively large eye, with horizontal diameter 21.01–32.63% in head length. Snout length (20.33–32.41% of HL) similar to eye diameter. Mouth inferior and slightly arched, without a conspicuous horny blade on lower lip. Dorsal and anal fins similar in size, both with seven branched rays, rarely eight, and with outer margin slightly convex. Origin of dorsal fin located above the end of pelvic fins or slightly posterior to this. Caudal fin with slightly rounded lobes. Numerous and medium-size scales, with a pigmented and complete lateral line. Short and numerous gill-rakers, along the entire gill arch. Six/five smooth and knife-shaped pharyngeal teeth, in one row.

Pigmentation pattern. In 70% ethanol the specimens of the type-series appear light brown on head, dorsum and upper flanks with blackish pigmentation; lateral line pigmented; creamy white on ventral region; fins greyish and sometimes with disperse pigmentation.

Distribution. C. almacai sp. nov. inhabits the southwestern Portuguese drainages Mira, Arade and the smaller Bensafrim (the population from Bensafrim drainage was assigned to the new taxon through molecular markers analysis; Mesquita et al. unpublished data) (Fig. 1).

Etymology. The species is dedicated to Professor Carlos A l m a ç a , for his long and outstanding contributions to the study of differentiation patterns and evolutionary processes of Euro-Mediterranean cyprinids.

Remarks. The new species is a reophilic fish, inhabiting Mediterranean-type streams that have typical seasonal habitat heterogeneity, and low to medium flowing clear waters. The main life-history traits of *C. almacai* sp. nov. and the relationship between population dynamics and environmental variability were studied in detail by M a g a l h ã e s (2002) in a broader analysis of fish communities of the Mira drainage. The species has a short life-span living up to four years, and becomes sexually mature early at the age of two years, irrespective of sex. However, 81.5% of males are mature at 70–80 mm total length, whereas only 50% of females are mature at 80–90 mm total length. M a g a l h ã e s (2002) also noticed high fecundity, apparently strongly correlated with total body length, with estimates of up to 3999 eggs at 100 mm total length; egg size ranges from 1.12 to 1.43 mm and is independent of total body length. Spawning occurs early in the year, from January to April.

Conservation. Although *C. almacai* sp. nov. is locally abundant, its very limited range, coupled with a high dependence on the spatial heterogeneity and temporal dynamics of the lotic systems it inhabits, increases the species' vulnerability to anthropogenic activities. Therefore, habitat homogenisation or destruction, by altered land use, damming, water extraction or introduction of exotic species, may significantly affect the species' survival in the future, justifying the inclusion of this species in the Critically Endangered category of IUCN Red List [IUCN Red List criteria B1ab(ii, iii, iv)c(iv)+2ab(ii, iii, iv)c(iv); ICN unpublished data, http://www.icn.pt/destaques/destaques_anexos/anexos_L_Ver/Classif_ Crit_Peixes.pdf; http://www.iucnredlist.org/info/categories_criteria.html].

Chondrostoma lusitanicum Collares-Pereira, 1980 [Fig. 3(b) and Table 1]
Holotype. MB 05-604 (M.B. c.1.1; C o l l a r e s - P e r e i r a 1980) April 1979, Xarrama R., Alcáçovas/Viana do Alentejo (38º21'39" N; 8º04'18" W), Sado drainage, southwest Portugal; L_S 82.5 mm.

Paratypes. 11 specimens, MB 05-605 (M.B. c.1.2-c.1.12; C o l l a r e s - P e r e i r a 1980) April 1979, Xarrama R., Alcáçovas/Viana do Alentejo (38º21'39" N; 8º04'18" W), Sado drainage, southwest Portugal.

Non-type material. 21 specimens, MB 05-1873 April 1979, Xarrama R., Alcáçovas/Viana do Alentejo (38º21'39" N; 8º04'18" W), Sado drainage, southwest Portugal; eight specimens MB 05-1871 October 1978, Freixo R., Pavia/Vimieiro (38º51'40" N; 7º57'00" W), Tejo drainage, central Portugal; 13 specimens MB 05-1872 April 1979, Freixo R., Pavia/Vimieiro (38º51'40" N; 7º57'00" W), Tejo drainage, central Portugal.

Diagnosis (Table 4). LLS (43)45–49(51) [46]; LLSA (9.5)10–11 [10.5]; RPT 5–6 [5].

Description. DR III (6)7(8) [7]; AR III (6)7–8 [7]; LLS (43)45–49(51) [46]; LLSA (9.5)10–11 [10.5]; LLSB 3.5–4.5(5) [4]; GR 27–36(41) [32]; LPT 6 [6]; RPT 5–6 [5]. Maximum total body length of 151 mm (R o d r i g u e s 1993), with relatively narrow, moderately laterally compressed body and rounded abdomen. Head relatively small, with

Fig. 3. Lateral view of (a) *Chondrostoma almacai* sp. nov. (holotype, 79.9 mm L_S, MB 05-1870/21) and (b) *Chondrostoma lusitanicum* (holotype, 82.5 mm L_S, MB 05-604).

length 20.54–25.28% in standard length; head depth about 1.6 times greater than CPD. Eyes small, inferior arched mouth, without a conspicuous horny blade on lower lip. Dorsal and anal fins similar in size, generally with seven branched rays in dorsal and seven or eight in anal, both with slightly convex profile; origin of dorsal fin located above end of pelvic fins or slightly posterior to this; caudal fin with slightly rounded lobes. Scales numerous and of medium-size, with distinct and complete lateral line; small and numerous gill rakers, along entire gill arch; six/five or six/six smooth and knife-shaped pharyngeal teeth, in one row.

Distribution. Chondrostoma lusitanicum occurs in the Tejo drainage (only in the most western tributaries and with a very patchy distribution), in the Sado drainage, and in some small western littoral drainages (Lizandro, Samarra, Colares and Ossos, according to S o u s a - S a n t o s 2001) (Fig. 1).

Table 4. Diagnostic characters between *C. almacai* sp. nov. and *C. lusitanicum*.

Characters	*C. almacai* sp. nov.	*C. lusitanicum*
Number of lateral line scales	‾=49.34 (45.00–53.00)	‾=47.25 (43.00–51.00)
Number of scales above lateral line	‾=11.05 (10.50–12.00)	‾=10.41 (9.50–11.00)
Number of right pharyngeal teeth	‾=4.99 (4.00–5.00)	‾=5.35 (5.00–6.00)
Head depth % of standard length	‾=16.01 (14.78–18.19)	‾=16.98 (15.90–19.59)
Snout length % of head length	‾=24.97 (20.33–32.41)	‾=22.57 (15.09–26.73)
Allozyme diagnostic locus	PGDH *50 and *75	*PGDH *100* and *125*

Discussion

The morphological differentiation between *C. almacai* sp. nov. and *C. lusitanicum* reported here is in accordance with the previous data described by C o l l a r e s - P e r e i r a (1983) and R o d r i g u e s (1993) for the Arade and Mira populations, in their comparative analyses with Tejo and Sado populations and the Samarra population, respectively. *C. almacai* sp. nov. and *C. lusitanicum* can be distinguished by a combination of several meristic and morphometric features (see *Diagnosis* for *C. almacai* sp. nov.). The most informative characters are the number of scales above and on the lateral line, the number of right pharyngeal teeth, the snout length and the head depth (Table 4). However, a large overlap exists between the two species when considering the overall morphology.

Nevertheless, molecular data strongly support the differentiation of the new species *C. almacai*. According to mitochondrial DNA data, *C. almacai* sp. nov. and *C. lusitanicum*, along with *C. lemmingii* and *C. oretanum*, are clearly sister-species, defining a strongly supported monophyletic group (Z a r d o y a & D o a d r i o 1998, 1999, C a r m o n a et al. 2000, D u r a n d et al. 2003, D o a d r i o & C a r m o n a 2004). High levels of cytochrome *b* gene sequence divergence (5.3-6.3%; M e s q u i t a et al. 2001) were observed between *C. almacai* sp. nov. (Mira and Arade populations) and *C. lusitanicum* (Sado, Tejo and Samarra populations), comparable to the values found between other pairs of *Chondrostoma* species belonging to this monophyletic group (e.g. 4.2% between *C. lemmingii* and *C. oretanum*, 5.0% between *C. lemmingii* and *C. almacai* sp. nov., and 6.2% between *C. oretanum* and *C. almacai* sp. nov.; C a r m o n a et al. 2000, D o a d r i o & C a r m o n a 2003, 2004, D u r a n d et al. 2003), as well as between other Cyprinidae sister species (C o e l h o et al. 1998, D o a d r i o et al. 2002, D o a d r i o & M a d e i r a 2004). Furthermore, in what concerns nuclear markers, a previous allozyme study (C o e l h o et al. 1997b) has also shown high levels of genetic divergence, with the presence of two fixed allelic differences at the *PGDH* locus (Table 4), for the Mira and Arade *Chondrostoma* populations (now recognized as *C. almacai* sp. nov.). This is in accordance with the levels of differentiation found between other *Chondrostoma* species (C o e l h o 1992, C o e l h o et al. 1997a, G o l l m a n n et al. 1997, C a r m o n a et al. 2000, D o a d r i o & C a r m o n a 2003).

The patterns of genetic divergence observed in the small extreme southwestern drainages for the species of both genera *Chondrostoma* and *Squalius* have been related to geological events affecting the evolution and isolation of the river courses (B r i t o et al. 1997, M e s q u i t a et al. 2001, S a n j u r et al. 2003). Namely, the formation during the lower Pliocene (3.4–5.3 MY) of the mountains ("Serra do Caldeirão") that enclose the Mira and Arade drainages (F e i o 1952, T e r r i n h a 1998, D i a s 2001), indicates an early isolation of these southwestern drainages (B r i t o et al. 1997, M e s q u i t a et al. 2001, S a n j u r et al. 2003). Moreover, these populations are subjected to seasonal fluctuations in habitat conditions, typical of the circum-Mediterranean region. The occurrences of flooding events during winter and severe droughts in summer (complete drying up of long extensions of the river courses, that often become a series of isolated pools) cause an increase in fish mortality, and subsequent genetic bottlenecks are likely to be promoted, resulting in recurrent genetic drift processes (C o e l h o et al. 1995, 1997b, M e s q u i t a et al. 2001).

Although *C. lusitanicum* was listed as Rare in the Portuguese Red Data (SNPRCN 1991), the high current rate of decline of the populations, which were locally abundant two decades ago, recommends that this species as well as *C. almacai* sp. nov. should be listed as Critically Endangered [IUCN Red List Categories criteria: *C. almacai* sp. nov., B1ab

(ii, iii, iv)c(iv)+2ab(ii, iii, iv)c(iv); *C. lusitanicum*, A2ce+3ce+4ce; ICN unpublished data, http://www.icn.pt/destaques/destaques_anexos/anexos_L_Ver/Classif_Crit_Peixes.pdf; http://www.iucnredlist.org/info/categories_criteria.html]. The very restricted distribution ranges of both species, and the periodic extreme fluctuations in water flow (i.e., floods/ droughts), make these populations and ecosystems vulnerable to external interferences. Therefore, as previously suggested by M a g a l h ã e s (2002) and M e s q u i t a & C o e l h o (2002) it is extremely important to protect such riverine habitats, in particular the summer refugia for these species, and to implement specific legal actions preventing land use changes, damming, water extraction and the dispersal of exotic species.

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