



universität  
wien

# MASTERARBEIT

Titel der Masterarbeit

„Phenotypic diversity of the threespine stickleback  
*Gasterosteus aculeatus* (Teleostei: Gasterosteidae)  
in the Mediterranean Sea –  
populations of the Adriatic Drainage System“

Verfasser

Markus Rokop, BSc

angestrebter akademischer Grad

Master of Science (MSc)

Wien, 2012

Studienkennzahl lt. Studienblatt:

A 066 831

Studienrichtung lt. Studienblatt:

Masterstudium Zoologie UG2002

Betreuerin / Betreuer:

Univ. Doz. Dr. Harald Ahnelt







## TABLE OF CONTENTS

ABSTRACT.....	1
ZUSAMMENFASSUNG .....	2
INTRODUCTION .....	3
SYSTEMATIC POSITION .....	3
DISTRIBUTION.....	3
ECOLOGY .....	4
MORPHOLOGY .....	5
ADRIATIC SEA BASIN .....	6
GENETICS .....	8
AIMS.....	9
MATERIAL AND METHODS .....	10
NERETVA SAMPLING SITES .....	12
DATA ACQUISITION.....	16
STATISTICAL ANALYSIS .....	17
RESULTS .....	19
GEOMETRIC MORPHOMETRICS.....	19
DISTANCE RATIOS .....	28
DISCUSSION.....	33
CONCLUSIONS.....	39
REFERENCES .....	40
ACKNOWLEDGEMENTS.....	46
CURRICULUM VITAE.....	47



## ABSTRACT

*Gasterosteus aculeatus* occurs in the Mediterranean Sea only in isolated coastal populations. During the Pleistocene the Adriatic Basin was affected repeatedly by changing sea levels. Thus Northern Adriatic freshwater populations of the threespine stickleback were isolated since at least the last glacial maximum. From these populations the Neretva System and Isonzo River form the most extreme points being the first and last isolated populations respectively. From these two systems, together with reference populations from Iznik Lake in Turkey and Mulargia in Sardinia, a total of 208 *G. aculeatus* specimens were examined with geometric morphometric methods to analyse variation of body shapes between populations as well as within one freshwater system, the Neretva System. The populations were analysed with relative warp analyses as well as with canonical variate analyses. Also the extent of sexual dimorphism on body shape and the state of parasitism with *Schiostocephalus solidus* and its influence on body shape is noted. Further the usage of a ratio of two distances (distance between the first/second and third dorsal spine, length of the dorsal fin) for sex determination is investigated. Body shape differences are not so much found between the Adria populations but mostly found within the Neretva System. Sexual dimorphism in body shape, especially in head morphology, is the highest source of variation and found in all populations. Only one population within the Neretva System is highly parasited and the body shape of these specimens is intermediate to not parasited males and females. The usage of one trait like a ratio of two distances to classify specimen as male or female is not advisable since sex dimorphism in body shape is comprised of many single small traits. The results of this study are mirrored by already existing genetic studies, thus complementing the picture of the populations of the Adriatic Drainage System.

## ZUSAMMENFASSUNG

*Gasterosteus aculeatus* tritt im Mittelmeer nur in isolierten, küstennahen Populationen auf. Während des Pleistozäns war das Adria Becken wiederholt durch schwankende Meeresspiegel Niveaus starken Veränderungen unterworfen. Daher sind die Süßwasserpopulationen des dreistacheligen Stichlings in der Nordadria zumindest seit der Maximalphase der letzten Eiszeit isoliert. Das Neretva System und der Fluss Isonzo stellen die extremsten Punkte dieser Region dar, nämlich jeweils die erste beziehungsweise die letzte isolierte Population. Aus diesen zwei Systemen, gemeinsam mit Referenz-Populationen vom Iznik See in der Türkei und Mulargia auf Sardinien, wurden insgesamt 208 *G. aculeatus* Individuen mit Methoden der geometrischen Morphometrie untersucht um die Varianz der Gestalt des Körpers zu untersuchen, sowohl zwischen Population als auch innerhalb einer Population, dem Neretva System. Die Populationen wurden sowohl mit Relative Warp Analysen als auch mit Canonical Variate Analysen untersucht. Auch wurde der Grad des Sexualdimorphismus in der Körpergestalt festgestellt, wie auch den Befall mit dem Parasiten *Schistocephalus solidus* und dessen Auswirkung auf die Körpergestalt. Ebenso wurde untersucht, inwieweit das Verhältnis von zwei Distanzen (Distanz zwischen ersten/zweiten und dritten Dorsalstachel, Länge der Rückenflosse) verwendbar ist um das Geschlecht zu bestimmen. Die Unterschiede in der Körpergestalt sind nicht so sehr zwischen den Populationen zu finden, sondern eher innerhalb des Neretva Systems. Sexualdimorphismus in der Körpergestalt, besonders am Kopf, stellt die größte Variation dar und findet sich in allen Populationen. Nur eine Population des Neretva Systems war stark vom Parasit *Schistocephalus solidus* befallen, wodurch die Körpergestalt der befallenen Individuen eine Mittelform zwischen nicht befallenen Männchen und Weibchen annahm. Es ist nicht ratsam nur ein Merkmal, wie zum Beispiel das Verhältnis zweier Distanzen, zu verwenden um das Geschlecht eines Individuums zu bestimmen, da der Sexualdimorphismus der Körpergestalt aus vielen einzelnen Merkmalen zusammengesetzt ist. Die Ergebnisse dieser Studie spiegeln die Ergebnisse bereits existierender genetischer Studien wieder, wodurch das Bild der Populationen des Adria Eizugsgebietes ergänzt wird.



## INTRODUCTION

### SYSTEMATIC POSITION

The systematic position of *Gasterosteus aculeatus* is described in Nelson (2006) and summarized here.

*Gasterosteus aculeatus*, as part of the Class Actinopterygii, is placed in the Division Teleostei and in there is positioned in the Superorder Acanthopterygii. Within this Superorder one of the Orders is the Order Gasterosteiformes. The Gasterosteiformes have two suborders, the Syngnathoidei and the Gasterosteioidei. The Gasterosteioidei contain four families, the Hypoptychidae, the Aulorhynchidae, the Indostomidae and the Gasterosteidae. Within the Gasterosteidae five genera exist, one of them is *Gasterosteus*. There are now only two accepted species within the generum. One is *Gasterosteus wheatlandi* Putnam, 1866, and the other is *Gasterosteus aculeatus* Linnaeus, 1758 (Paepke, 2002; Nelson, 2006). The genus *Gasterosteus*, comprised of *G. aculeatus* and *G. wheatlandi*, is viewed as a monophyletic sister lineage to all other species within the Gasterosteidae (Kawahara et al., 2009). Nelson (2006) views *G. aculeatus* as a species complex rather as a single species due to extensive phenotypic variation.

Kotellat (1997) discussed the distribution of *G. aculeatus* within Europe and suggested a classification in four different species, based upon the distribution patterns in Europe. He suggested *G. gymnurus* Cuvier, 1829 for the western and southern populations of *G. aculeatus*. This has been considered and discussed by Kleinlerchner et al. (2008) and now *G. gymnurus* is still used in some works (Vila-Gispert, Alcaraz & García-Berthou, 2005; Magalhães et al., 2007; Filipe et al., 2009; Maceda-Veiga et al., 2010; Vizi & Vizi, 2010; Sánchez-Hernández et al., 2011). Since the majority of the studies published about the threespine stickleback refer to *G. aculeatus* as the threespine stickleback (see References in this study), the threespine stickleback is referred to as *G. aculeatus* in this study too.

### DISTRIBUTION

The threespine stickleback, *Gasterosteus aculeatus*, has a widespread distribution throughout the Holarctic and is found on all three continents in the northern hemisphere (Wootton, 1984; Bell & Foster, 1994b; Bell & Andrews, 1997; Paepke, 2002), mainly in cold and temperate regions (Münzing, 1963; Paepke, 2002). In Northern America the threespine stickleback is found on the Pacific coast from Alaska to Southern California in

marine and freshwater habitats, while on the Atlantic coast habitats, marine and freshwater, are only found north of Maine (Münzing, 1963; Wooton, 1984). In Asia the south most distribution is Japan and Korea and there only in coastal areas (Wooton, 1984).

Within Europe *G. aculeatus* has settled around coastal regions from the Barent Sea, across Iceland to Great Britain and Ireland, as well as Scandinavia, the North Sea and the Baltic Sea to North Europe (Paepke, 2002). In Central Europe *G. aculeatus* was introduced in many freshwater areas, like in Austria, Czech, Slovakia, Hungary and Switzerland (summarized in Paepke, 2002). The southern border of the distribution is the northern coast of the Mediterranean Sea, for example Spain, Italy and the Balkan peninsula (Münzing, 1963; Wooton, 1984; Paepke, 2002). The eastern distributions reach as far north as the White Sea, while the most eastern habitats are the west coast of the Black Sea and as far as the River Dnieper (Münzing, 1963; Wooton, 1984).

Within the Mediterranean Sea no marine populations are present, only isolated freshwater populations in rivers flowing into the Mediterranean, like in Spain, Italy, the Balkan Peninsula and as far as Turkey (Münzing, 1963; Wooton, 1984; Paepke, 2002). *G. aculeatus* has been collected in Italy since the mid of the 19th century in more than 30 localities, on the Italian Peninsula and on Sardinia Island (Bianco, 1980). Along the Balkan Peninsula *G. aculeatus* is reported to be present in Croatian freshwater systems, like River Neretva and its tributaries (Karaman, 1928; Mrakovcic, Misetec & Povz, 1995; Zanella, 2009; Zanella et al., 2009; DeFaveri et al., 2012). Also in Turkey *G. aculeatus* has been reported in freshwater habitats, like Iznik Lake (Münzing, 1962; Özuluğ, Altun & Meriç, 2005; Tarkan et al., 2006).

## ECOLOGY

*Gasterosteus aculeatus* was widely investigated for its ethology, especially courtship and parental behaviour (for a summary see Bell & Foster, 1994b; Hopkins, Moss & Gill, 2011; Morrell et al., 2012; Snowberg & Bolnick, 2012). However, *G. aculeatus* shows different ecological forms and can be present in a wide range of different habitats (Reimchen, Stinson & Nelson, 1985; Paepke, 2002; Clavero, Pou-Rovira & Zamora, 2009). These include marine, brackish and freshwater habitats (Bell & Foster, 1994b). Populations found in marine habitats are believed to be the primitive form (Paepke, 2002; Furin, von Hippel & Bell, 2012), from which other forms, anadromous and freshwater,

evolved (Bell & Foster, 1994b; Bell & Andrews, 1997; Aguirre & Akinpelu, 2010; Furin et al., 2012). Genetics as well as systematics, and zoogeography indicate that freshwater habitats have been repeatedly and independently colonized by anadromous sticklebacks (Bell & Foster, 1994b; Bell & Andrews, 1997), thus forming populations in low flowing streams, large rivers, lakes, ponds and swamps (Reimchen et al., 1985; Bell & Andrews, 1997).

Also within one habitat two different populations of *G. aculeatus* can occur sympatric, by having a pelagic or benthic lifestyle (Baumgartner, 1992). This affects the body form because of different environmental constraints (Campbell, 1985; Spoljaric & Reimchen, 2007; Aguirre, 2009), especially feeding (Caldecutt & Adams, 1998; Sánchez-Hernández et al., 2011). Predation is also known to have a large influence on morphological traits, especially defensive structures like lateral plates, spines and elements of the pelvic girdle (Gross, 1978; Reimchen, 1983; Reimchen, 1992), which together are called the defensive complex (Ahnelt et al., 1998; Ahnelt et al., 2006b).

## MORPHOLOGY

The threespine stickleback shows a high variation in external morphology (Bell & Foster, 1994b). Marine and anadromous sticklebacks are rather uniform in morphological traits, while divergences of body shape and morphological structures are prominent in freshwater population (Campbell, 1985; Reimchen et al., 1985; Spoljaric & Reimchen, 2007; Aguirre, 2009; Webster et al., 2011). One of the most prominent variations in threespine sticklebacks is the varying expression of the bony plates on the lateral side, the lateral plates. These plates, if maximally expressed, form a continuous row posterior of the head to the end of the caudal peduncle. On the caudal peduncle the plates, then called keel plates, form a distinct keel (Wootton, 1984; Bell & Foster, 1994b). The present number of lateral plates varies greatly between and within populations, from continuous row of plates to a few missing plates up to a complete reduction of lateral plates, spines and reduced elements of the pelvic girdle (Penczak, 1965; Campbell, 1985; Klepaker, 1995; Kitano et al., 2008; Barrett, 2010). Thus four different plate morphs are recognized, following the nomenclature of Ziuganov (1983), Banbura & Bakker (1995) and Ahnelt, Muerth & Lunardon (2006a): completely plated, partially plated, low plated and low plated with keel. Marchinko & Schluter (2007) distinguish only two plate morphs at all, a

reduced morph, for partially and low plated, and a complete morph, independent of the number of lateral plates or the presence of a keel.

The distribution of the different plate morphs across Europe seems to follow a pattern which indicates a correlation with the different ecological constraints from their habitats (Bell & Foster, 1994b; Paepke, 2002). Populations in marine coastal habitats as well as the anadromous form tend to be monomorphic completely plated (Wootton, 1984; Bell & Foster, 1994b), while freshwater populations can be polymorphic with a mixture of different plate morphs (Münzing, 1963), or monomorphic low plated (Wootton, 1984; Bell & Foster, 1994b).

Especially populations in the Mediterranean region are low plated (Münzing, 1963; Wootton, 1984; Bell & Foster, 1994b; Paepke, 2002), but also low plated with keel, earlier described as partially plated, occur (Münzing, 1962).

The reasons for such distributions are several: for example one fact might be the difference of burst and prolonged swimming styles, as discussed by Taylor & McPhail (1986), another might be the influence of temperature and salinity on different osmoregulation systems, as summarized in Paepke (2002).

Other divergent traits studied in *G. aculeatus* are mostly parts of the defensive complex, a flexible and bony structure comprised of 20 bony elements which encompass the abdominal region of the body to protect the fish from injuries by toothed predators (Gross, 1978; Reimchen, 1983; Reimchen, 1992; Ahnelt et al., 1998; Ahnelt et al., 2006b).

Next to the mentioned traits, overall body form is highly divergent throughout all populations and especially differences between male and female sticklebacks are prominent (Aguirre et al., 2008; Aguirre & Akinpelu, 2010). Female sticklebacks are generally larger in body length (Aguirre et al., 2008), while male sticklebacks have a larger head length, a larger snout length and a larger eye diameter (Aguirre & Akinpelu, 2010).

## ADRIATIC SEA BASIN

The Adriatic Sea basin was repeatedly subjected to lowered sea levels in the past. During glaciations in ice ages the sea level was lower across the globe. During the last glacial maximum (LGM), approximately 18.000-26.000 years BP, the sea level was about 120m lower than today (Fairbanks, 1989; Peltier & Fairbanks, 2006). During the LGM the

## Phenotypic diversity of *Gasterosteus aculeatus* in the Mediterranean Sea

North-Adriatic Sea basin was devoid of marine water (Fig. 1), and the River Po was extended until the now middle Adriatic where it terminated into the Fossa Meso-Adriatica (Bianco & Miller, 1990), which formed a freshwater lagoon (van Straaten, 1971). The last tributary to this river system was Krka River in Croatia, while the northernmost was Isonzo River in Italy (Bianco & Miller, 1990). The Neretva System was not part of the Freshwater System 18.000 years BP, but was probably connected during a glacial maximum at least 340.000 years BP when the sea level was approximately 240m lower than today (Rabineau et al., 2006). With rising temperatures an increase in sea level occurred and over the time of several thousand years the North-Adriatic basin changed from freshwater to marine conditions, isolating freshwater systems (Correggiari et al., 1996). With an increased water temperature, the marine and anadromous form of *G. aculeatus* was probably extinct in the Mediterranean Sea (Münzing, 1963; Paepke, 2002). Further genetic exchange was inhibited due to the lack of anadromous sticklebacks. Thus *G. aculeatus* shows a pan-Mediterranean distribution, typical for peripheral freshwater species (Bianco, Ahnelt & Economidis, 1996). Similar distributions in isolated freshwater systems are observed with the freshwater gobiid *Padogobius martensii* (Günther 1861) (Bianco & Miller, 1990) and also in continental Aegean Anatolia and eastern

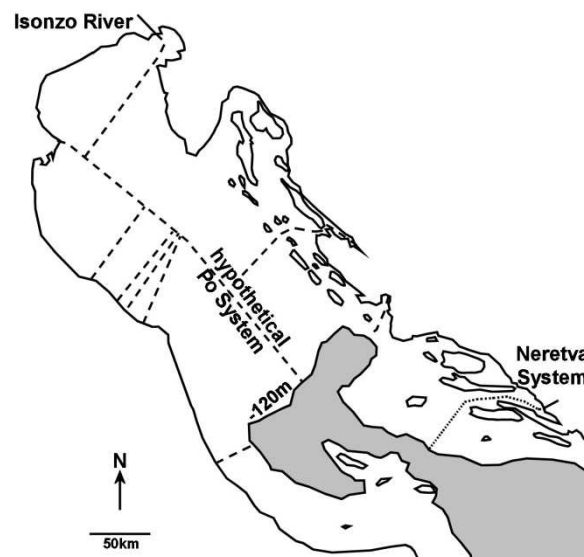


Fig. 1. Adriatic Sea during the LGM with the 120m lowered sea level (grey); also the hypothetical Po System (dashed lines) in the North Adriatic Basin is shown. Especially noted is the mouth of the Isonzo River as it is today. Also today's mouth of the Neretva System and its hypothetical outlet during the LGM (dotted line) is shown (modified after Bianco & Miller (1990) and Correggiari, Roveri & Trincardi (1996)).

Mediterranean within the genus *Knipowitschia* (Bianco et al., 1996). The genus *Knipowitschia* consists of primary-like freshwater fishes which show similar distribution patterns as primary freshwater fishes (Bianco et al., 1996). The distributions of *K. caucasica* (Berg 1916) is formed, among other factors, by Pleistocene glaciation and deglaciation events (Ahnelt, Bianco & Schwammer, 1995; Ahnelt, 2011), similar to the distribution of *G. aculeatus*.

## GENETICS

The genetic background of *Gasterosteus aculeatus*, especially the basis of the plate morphs but also the genetic relationships, has puzzled scientists for decades and much work was done all over the world to unravel this puzzle (e.g.: McKinnon & Rundle, 2002; Albert et al., 2008; Sharpe et al., 2008; Schluter & Conte, 2009; Barrett, 2010). Since the expression of the lateral plates and the keel have a genetic basis, models for the inheritance of lateral and keel plates were created, to try to explain the observed plate morph distributions (Ziuganov, 1983; Banbura & Bakker, 1995). Recent studies draw more accurate pictures of the genetic background and focus on quantitative trait loci (QTL). For example, the *Ectodysplasin (Eda)* allele is responsible for the expression and evolution of the low plated morph in freshwater habitats (reviewed in Barrett, 2010). Based upon the *Eda* allele Colosimo et al. (2005) provided the transporter hypothesis by which the low plated morph is present in marine populations as standing variation and breaks through if *G. aculeatus* settles in freshwater habitats.

Considerable work has been done to resolve the genetic relationships of *G. aculeatus* populations in Europe based upon mtDNA and microsatellite data (Mäkinen, Cano & Merilä, 2006; Cano et al., 2008; Mäkinen & Merilä, 2008; DeFaveri et al., 2012).

Freshwater populations in the Adriatic Drainage System have repeatedly been the target of genetic analyses (Mäkinen et al., 2006; Cano et al., 2008; Mäkinen & Merilä, 2008; DeFaveri et al., 2012), who repeatedly grouped Adriatic populations separately of the rest of Europe based mainly on mtDNA or microsatellites. DeFaveri et al. (2012) set the northern border of the Adriatic Lineage of *G. aculeatus* south of Krka River, thus counting all *G. aculeatus* populations within the Adriatic Drainage System north of Krka River to the European Lineage. The Adriatic Lineage was further divided in a Skadar Clade and a Neretva Clade (DeFaveri et al., 2012).

## Phenotypic diversity of *Gasterosteus aculeatus* in the Mediterranean Sea

The *G. aculeatus* populations of the Balkan Peninsula are sometimes referred to as ancient glacial refugia (Mäkinen & Merilä, 2008) and even deemed as protective conservation units (Cano et al., 2008).

Morphological studies about Mediterranean populations are rare. Some works exist from Spain (Lobon-Cervia, Penczak & de Sostoa, 1988; Hermida et al., 2005), France (Crivelli & Britton, 1987) and Italy (Bianco, 1980), but only few were done in the Adriatic System and especially in the Balkan Peninsula (Vuković & Prolić, 1966; Vuković & Kosorić, 1967; Zanella, 2009; Zanella et al., 2009). Rarely geometric morphometric methods were used to analyse *G. aculeatus* populations in the Balkan Peninsula. Also a lack of the combination of morphometric with genetic methods is observable. Since DeFaveri et al. (2012) uses similar populations from the Neretva System as used in this study, a comparison of genetic data with geometric morphometric data is possible.

### AIMS

After the LGM, freshwater populations of *Gasterosteus aculeatus* in the Adriatic System were gradually isolated during several thousand years with Isonzo River as the last isolated river. Since *G. aculeatus* starts breeding after one or two years (Foster, 1994) morphological differences, if present, between the populations of the Neretva System and Isonzo River might be the result of evolutionary change of several thousand generations. The tributaries of the River Neretva and the River Neretva itself possess different ecological conditions. There is a large body of literature on the adaptation of threespine stickleback body shape and other morphological traits to different environments (e.g.: Hagen & Gilberts, 1972; Giles, 1983; Reimchen et al., 1985; Crivelli & Britton, 1987; Baumgartner, 1992; Ahn & Gibson, 1999; Spoljaric & Reimchen, 2007; Sharpe et al., 2008; Clavero et al., 2009), but little is known about stickleback populations in the Adriatic System. In this study I investigate body shape differences between populations within the Neretva System using geometric morphometric methods.

It has been shown for several stickleback populations that male sticklebacks are smaller in standard length, have a larger head length, a larger snout length and a larger eye diameter relative to female sticklebacks (Caldecutt & Adams, 1998; Aguirre et al., 2008; Aguirre & Akinpelu, 2010). I assess sexual dimorphism in Neretva populations of the threespine stickleback and compare pattern and magnitude of sexual dimorphism across different populations.

## MATERIAL AND METHODS

A total of 208 *Gasterosteus aculeatus* specimens were examined. They originate from different freshwater systems (Fig. 2): the Neretva System in Croatia and in Bosnia Herzegovina, the River Isonzo in Italy, Mulargia on Sardinia Island in Italy, and the Iznik Lake in Turkey. Within the Neretva System, four sampling sites were used: Buna River, Hutovo Blato Wetland Canal, Neretva River and Norin River (Fig. 3).

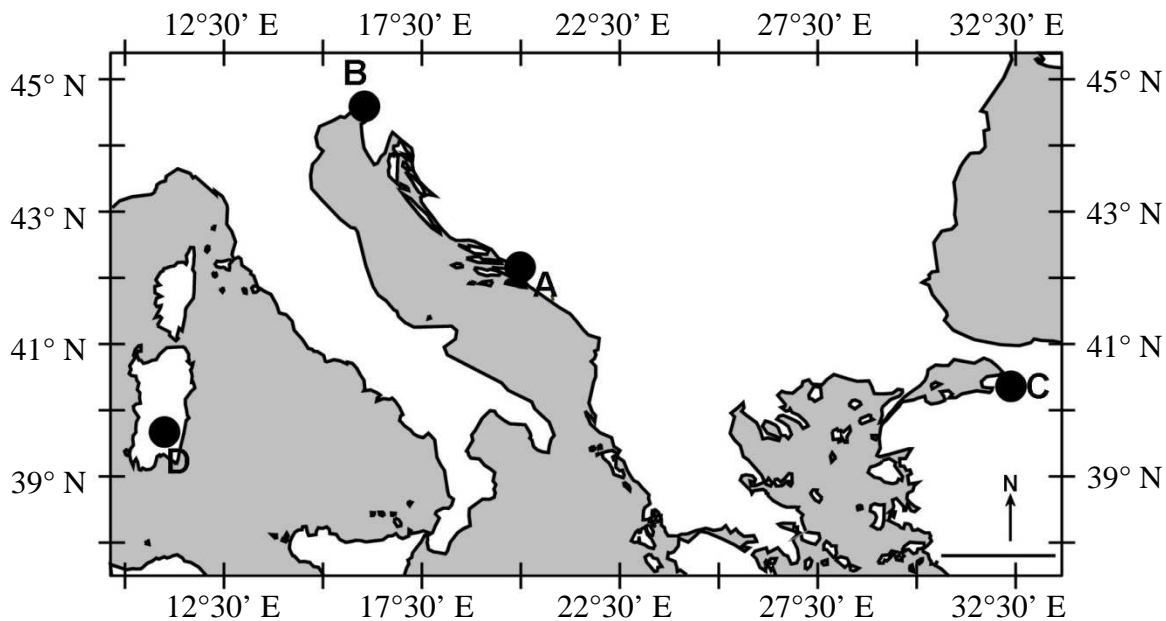


Fig. 2. Locations of *Gasterosteus aculeatus* sites in the North Mediterranean Sea. A: Neretva System in Croatia and in Bosnia and Herzegovina, B: Isonzo River near Monfalcone in Italy, C: Iznik Lake in Turkey, D: Mulargia on Sardinia Island in Italy. Scale bar is 200km.

Specimens from Iznik Lake and from Mulargia were from the Zoologisches Museum Hamburg, Germany. Specimens from the River Isonzo were from the Naturhistorisches Museum Wien, Austria. The specimens were preserved in 70% ethanol. Samples from the Neretva System were collected during surveys of the Institute of Oceanography and Fisheries in Split in May 2009, August 2009 and May 2010. The samples are deposited in the collection of Harald Ahnelt. All specimens have been preserved in 70% ethanol. Fresh caught specimens were euthanized with a water-clove oil emulsion (clove oil concentration: 100% *Eugenia caryophyllata*) and preserved in 4% formol. Afterwards, they were first transferred to 40% and then to 70% ethanol.



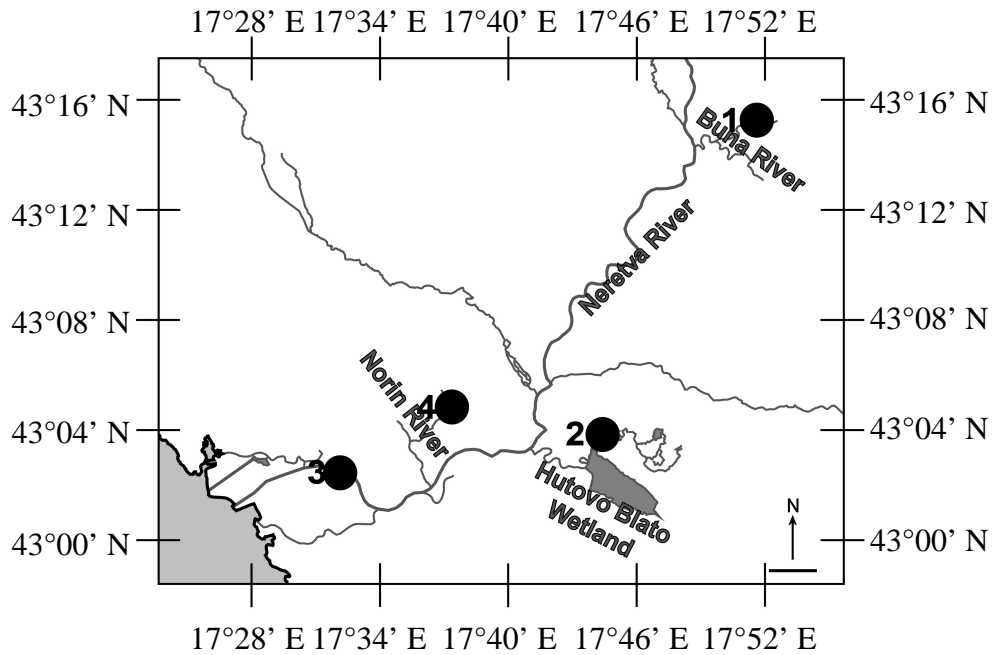


Fig. 3. Sampling sites of *Gasterosteus aculeatus* in the Neretva System. 1: Buna River, 2: Hutovo Blato Wetland Canal, 3: Neretva River near Komin, 4: Norin River near Vid. Scale bar is 3km.

Only adult (standard length (SL)  $\geq 30.0$  mm) specimens were used for analyses. Maturity of *G. aculeatus* is defined as the point, when no additional lateral plates are developed (Bell, 1981). There are different opinions in the literature when this point is achieved. Wooton (1984) describes that adult *G. aculeatus* are typically 35-80 mm in total length. Bell (1981) reports that the point of maturity for completely and low plated specimens differs by 8.8 mm in standard length (completely: 30.3 mm; low: 21.5 mm). If the caudal fin in *G. aculeatus* is not more than 5.0 mm in length, the standard length point of adulthood of Wooton (1984) and the completely morph of Bell (1981) are similar. Since in this work only low plated morphs were used, a specimen with a SL of 30.0 mm or more certainly completed its development of number of lateral plates, according to Bell's (1981) and Wooton's (1984) above mentioned criteria. Thus for this study the threshold of maturity is set to 30.0 mm SL.

The sex of all specimens was determined by gonad inspection. For this, a cut was made on the right body side caudal to the last lateral plate under a binocular. Dissecting and determining the sex of a specimen was always the last procedure after all measurements.

From the Neretva System a total of 105 specimen (male: n=49, female: n=56; SL: 30.0-65.0mm) were collected. Within the Neretva System Buna River was represented with 33 specimens (male: n=14, female: n=19; SL: 30.0-55.0mm), Hutovo Blato Wetland with 8 specimens (male: n=8, no females present; SL: 35.0-45.0mm), Neretva River with 27 specimens (male: n=21, female: n= 6; SL: 30.0-60.0mm) and Norin River was represented with 37 specimens (male: n=6, female: n= 31; SL: 30.0-65.0mm). From Isonzo River a sample with 17 specimens (male: n=13, female n=4; SL: 30.0-70.0mm) was available. The Iznik Lake sample had 76 specimens (male: n= 12, female: n=64; SL: 40.0-65.0mm). Mulargia had a sample size of 10 specimens (male: n=7, female: n=3; SL: 45.0-55.0mm).

## NERETVA SAMPLING SITES

### Buna River

Coordinates: 43°14'46" N, 17°51'21" E

Above sea level: 38m



Fig. 4. Buna River sampling site.

Buna River is a side arm of Neretva River, approximately 51.3km upstream. The sampling site was located 3.3km upstream in Buna River. The river was low to medium in current speed. At the river bank areas with very low current speed were found (Fig. 4). The river bed was mostly mud with large dense patches of algae and submerged weed and trees. Especially at the river bank the trees provided shading. The sampling was done in

## Phenotypic diversity of *Gasterosteus aculeatus* in the Mediterranean Sea

1.0-1.3m depths at low current speed areas with dense vegetation or in open areas directly next to vegetation. Buna River was sampled on three different days: one in May 2009, one in August 2009, and one in May 2010. Water temperature ranged from 11.2-15.9 °C, while Salinity was 0.1-0.2ppt. Oxygen levels were 5.22-8.7mg/l, which are 62.8-86.5% O<sub>2</sub> (Tab. 1).

### Hutovo Blato Wetland

Coordinates: 43°03'51" N, 17°45'19" E

Above sea level: 2m



Fig. 5. Hutovo Blato Wetland sampling site.

The sampling site in the Hutovo Blato Nature Park in Bosnia and Herzegovina was a small canal, a side arm of Krupa River. Krupa River inserts on the left side in Neretva River 24.6km upstream. The sampling site has a distance to Neretva River of 9.1km. The canal has a very low water current and is highly vegetated (Fig. 5). Dense patches of water plants as well as floating plants were located near the river bank. The canal bed consisted mainly of mud. Since the canal was used for boat travelling, the middle of the canal was much deeper than near the river bank and was freer of vegetation. Thus the water surface was not shaded by trees. Sampling was done near the river bank in 0.5-1.0m water depth near and under plants. Sampling was conducted two times, once in May 2009 and once in August 2009. Water temperature was 17.7-19.3°C and salinity 0.2-0.7ppt. Oxygen levels were 1.90-5.83mg/l.

Neretva River

Coordinates: 43°02'29" N, 17°31'56" E

Above sea level: 0m



Fig. 6. Neretva River sampling site.

Along the right river bank of the Neretva River, approximately 7.9km upstream, the city Komin is located. There the Neretva is approximately 115m wide, has a water depth of far more than 2m and has a medium to fast current. The river bank is steep and artificially regulated, although dense algae patches still occur (Fig. 6). One sampling was done in May 2009 by hand net on the right river bed in and around algae patches. The second sampling in May 2010 was conducted by a fisherman during the morning fish catching. The sticklebacks have been a by-catch. Ecological factors were assessed only in May 2009. Water temperature was 16.8°C and salinity 1.2ppt. Oxygen level was at 5.40mg/l and 68.3%.

Norin River

Coordinates: 43°04'49" N, 17°37'44" E

Above sea level: 1m

Approximately 16.1km upstream, the River Norin runs into the River Neretva. If followed upstream for 7.9km the town of Vid can be found. In this area the River Norin is highly regulated and water current is low (Fig. 7). The river bed consists mostly of gravel with patches of algae. No shade is provided by the surrounding vegetation because of the



Fig. 7. Norin River sampling site.

controlled environment. The only shade is given by bridges across the river. Sampling has been conducted in August 2009 and May 2010. Both times it was conducted in algae and plant patches near a stone bridge in 0.5m depth. The water temperature was 16.6-17.5°C with 0.3-0.4ppt salinity. The oxygen levels were 5.72-5.82mg/l.

Tab. 1: Ecological data of the sampling sites from each collection date: water temperature in degrees celsius (Temp), levels of salinity in parts per trillion (Sal), levels of oxygen in milligram per liter and in percent.

<b>Population</b>	<b>Temp [°C]</b>	<b>Sal [ppt]</b>	<b>O<sub>2</sub> [mg/l]</b>	<b>O<sub>2</sub> [%]</b>
<i>Buna River</i>				
27.05.2009	15.9	0.2	8.70	86.5
27.08.2009	15.5	0.2	5.22	
26.05.2010	11.2	0.1	7.15	62.8
<i>Hutovo Blato Wetland</i>				
26.05.2009	19.3	0.2	5.83	69.8
29.08.2009	17.7	0.7	1.90	
<i>Neretva River</i>				
26.05.2009	16.8	1.2	5.40	60.5
<i>Norin River</i>				
28.08.2009	16.6	0.4	5.82	
25.05.2010	17.5	0.3	5.72	62.5



DATA ACQUISITION

Tab. 2: Used Landmarks and their positions, modified after Walker (1997). Landmark types follow Bookstein (1991) (Bookstein, 1991) (Bookstein, 1991). Nr. = Landmark Number; Definition = exact description of position; Type = Landmark Type 1, 2 or 3; sl = sliding landmark

Nr.	Definition	Type
1	most anterior point of praemaxillare and dentale touching	2
2	anterior insertion point of first dorsal spine at dorsal midline	1
3	anterior insertion point of second dorsal spine at dorsal midline	1
4	anterior insertion point of third dorsal spine at dorsal midline	1
5	anterior base of first dorsal fin ray	1
6	anterior base of last dorsal fin ray	1
7	insertion point of the fin membrane of the caudal fin at dorsal midline	1
8	mid point of posterior edge of the caudal peduncle	3
9	insertion point of the fin membrane of the caudal fin at ventral midline	1
10	anterior base of last anal fin ray	1
11	anterior base of first anal fin ray	1
12	anterior insertion point of anal spine at ventral midline	1
13	posterior tip of ventral processus of pelvic girdle	2
14	anterio-ventral base of left ventral spine	2
15	most posterior edge of ectocoracoid	3
16	anterior tip of ectocoracoid	3
17	posterior edge of lower jaw	3
18	most dorsal edge of orbit	3
19	most anterior edge of orbit	3
20	most ventral edge of orbit	3
21	most posterior edge of orbit	3
22	dorsal start of operculum membrane	1
23	most dorsal edge of ectocoracoid	3
24-28	dorsal curve of head at dorsal midline	sl
29-30	dorsal curve of caudal peduncle at dorsal midline	sl
31-32	ventral curve of caudal peduncle at ventral midline	sl
33-35	ventral curve of pelvic and pectoral girdle at ventral midline	sl
36-37	ventral curve of throat at ventral midline	sl
38-39	ventral curve of lower jaw at ventral midline	sl

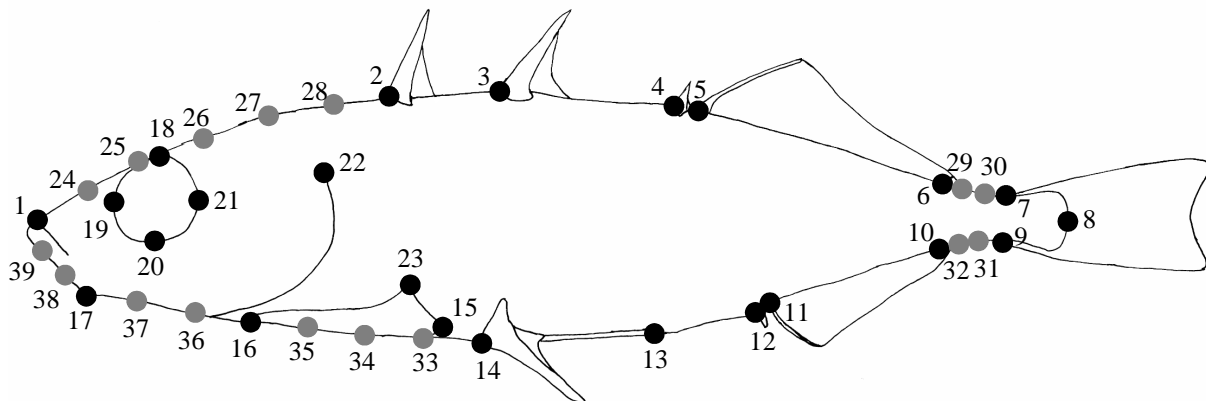


Fig. 8. Landmarks used for Geometric Morphometrics with *G. aculeatus* specimens; LM 1-23 normal Landmarks (black), LM 24-39 Semi-Landmarks (gray).

Pictures were taken with an Olympus *E-3* digital Camera with a 50mm 1:2 Macro Objective. The Camera was mounted on a Repro-Stativ. Pictures from adult specimens ( $SL \geq 30.0$  mm) were taken at a distance of 55cm. The specimens were put on a self-made wooden pedest. The wooden beams where the specimens were put on were adjustable for the length of the specimens. The pedest with the specimen was illuminated with four 40W opal light bulbs. On all pictures a scale bar and the specimen's ID was present. Pictures taken from the left side were used for further statistical analyses. Landmark digitalization was done with the program tpsDig, version 2.12 (Rohlf, 2008a). 39 Landmarks (Tab. 2) were set to describe the shape of the specimen. With tpsUtil, version 1.44 (Rohlf, 2009b) 16 of the 39 landmarks were defined as sliding landmarks (Fig. 8). This type of landmarks extend landmark-based statistics to curves and smooth surfaces (Mitteroecker & Gunz, 2009), providing a more comprehensive description of form and shape of the object.

## STATISTICAL ANALYSIS

Landmark digitalization was conducted with tpsDig, version 2.12 (Rohlf, 2008a) and relative warp analyses were done in tpsRelw, version 1.46 (Rohlf, 2008b). Regressions on size and sex were conducted with tpsRegr, version 1.37 (Rohlf, 2009a), and analyses of centroid size were done with tpsSpline, version 1.20 (Rohlf, 2004).

Since it is not possible to create plots with grouped areas in tpsRelw, the score matrix of the relative warps as well as Centroid Size and further information, like sex and state of parasitism, were imported into PAST, version 1.91 (Hammer, Harper & Ryan, 2001). To achieve this, the necessary data, which were saved as a .nts file, was converted to a .csv file with the nts2csv program (Cavalcanti, 2008) and opened in MS Excel for Mac 2011, version 14.0.2. There the columns were renamed and the data was copied to PAST, where a grouped plot could be drawn.

For further analyses certain distances between Landmarks were calculated following the formula based upon Pythagoras' theorem:

$$x_{LMD} = \left( \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \right) \cdot x_{scale} \cdot 10$$

where  $x_{LMD}$  is the calculated landmark distance,  $x_1, y_1, x_2, y_2$  are the x and y coordinates for the landmarks respectively,  $x_{scale}$  is the scale factor for the picture and 10 is the factor to transfer from centimeter to millimeter.

The landmark distance ratio described in chapter 3.2 follows the formula:

$$LMD_{ratio} = \frac{x_{LMDa}}{x_{LMDb}}$$

where  $LMD_{ratio}$  is the calculated landmark distance ratio,  $x_{LMDa}$  is the first landmark distance and  $x_{LMDb}$  is the second landmark distance.



## RESULTS

### GEOMETRIC MORPHOMETRICS

A scatter plot of the first two relative warps of the sex-specific average shapes of the sticklebacks from four populations, Neretva System, Isonzo River, Iznik Lake and Mulargia, shows that the two Adria populations, Neretva System and Isonzo River, are more similar to Iznik Lake population than to Mulargia population (Fig. 2). The first relative warp (accounting for 42.0% of total shape variation) describes the differences between the outgroup Mulargia to the rest of the populations. These differences are mainly in body height (LM 3 to 14, 4 to 13), the lengths of the dorsal and anal fin (LM 5-6, 10-11), and in the form of the caudal peduncle (LM 6-10). The distance from the caudal tip of the ventral process to the anal spine (LM 12-13) and also the form of the head (LM 1, relative to LM 24 and LM 39) differs along the first relative warp (Fig. 2). The second relative warp (accounting for 39.7% of total shape variation) represents differences in body shape between male and female specimens. The mean values of male specimens have positive scores in all Adria populations. The mean values of female specimens of Adria populations have negative scores, except for Mulargia sticklebacks, where a low positive score is present (Fig. 2). Differences in body shape comprise the diameter of the eye (LM 18-21) and also the distance between the second and third dorsal spine (LM 3-4), the length of the ventral process (LM 13-14), anal fin length (LM 10-11), and the distance between anal spine and the tip of the ventral process (LM 12-13). Also the whole head form is affected (Fig. 2).

A scatter plot of the first two relative warps of the sex-specific average shapes of the sticklebacks from four populations of the Neretva System (Buna River, Hutovo Blato Wetland, Neretva River and Norin River) (Fig. 10), shows a distribution pattern mainly influenced by sex and the separation between Buna River population and the rest of the Neretva populations. The first relative warp (accounting for 68.4% of total shape variation) shows mainly body shape differences between male and female sticklebacks. These differences are located in the head and abdominal regions. Male sticklebacks have larger heads (LM 1-2, 16-17, 24-28 and 33-39) and larger eyes (LM 18-21). Thus in female sticklebacks the landmarks of the head region are closer to each other, than in male sticklebacks. The second main difference is that the abdomen is shorter in males. This fact mainly influences the length of the anal fin (LM 10-11) and the distance

between the tip of the ventral process and the anal spine (LM 12-13). Another difference is that in females the distance between the second and third dorsal spines (LM 3-4) is larger than in males.

The second relative warp (accounting for 18.5% of total shape variation) displays differences between Buna River specimens and the other three Neretva System populations (Fig. 10). Buna River sticklebacks have a larger body height (LM 2 to LM 14), smaller eyes (LM 18-21) and the dorsal spines are located closer together (LM 2-4). Also the ventral process (LM 10-11) and the anal fin (LM 13-14) are shorter than in other Neretva System populations. The relative position of the ventral process to the anal spine (LM 12-13) is different in Buna River sticklebacks (Fig. 10).

To achieve a better group separation for male and female subgroups of each investigated population a canonical variate analysis based upon the first 15 relative warps of the previously conducted relative warp analysis was calculated (Fig. 11). This was done for the Adria grouped populations as well as for the Neretva System populations.

The CVA of the Adria grouped populations reveals a clustering mainly influenced by sex (Fig. 11A). The first two canonical variates (CV 1: accounting for 62,0% of total shape variation; CV 2: accounting for 17,4% of total shape variation), split all populations (Neretva System, Isonzo River, Iznik Lake, Mulargia) in male and female clusters. Only female Neretva System specimens overlap in a rather large area with male specimens from all other populations. All other populations separate between male and female specimens. Clusters of female specimens show overlapping areas between the populations. The clusters of male specimens show no overlapping areas, except for male Neretva System specimens.

As in the relative warp analysis of Neretva System populations, the CVA shows several clusters in the first two canonical variates (CV 1: accounting for 54.6% of total shape variation; CV 2: accounting for 36.5% of total shape variation), reflecting population separations and sex (Fig. 11B). The population separation is mainly between Buna River sticklebacks and the rest of the Neretva System populations. Also the first relative warp is represented by sex differences. Thus the CVA clusters the Neretva System populations mainly by body shape differences, which separate Buna River population from the rest of the Neretva System populations and sex.

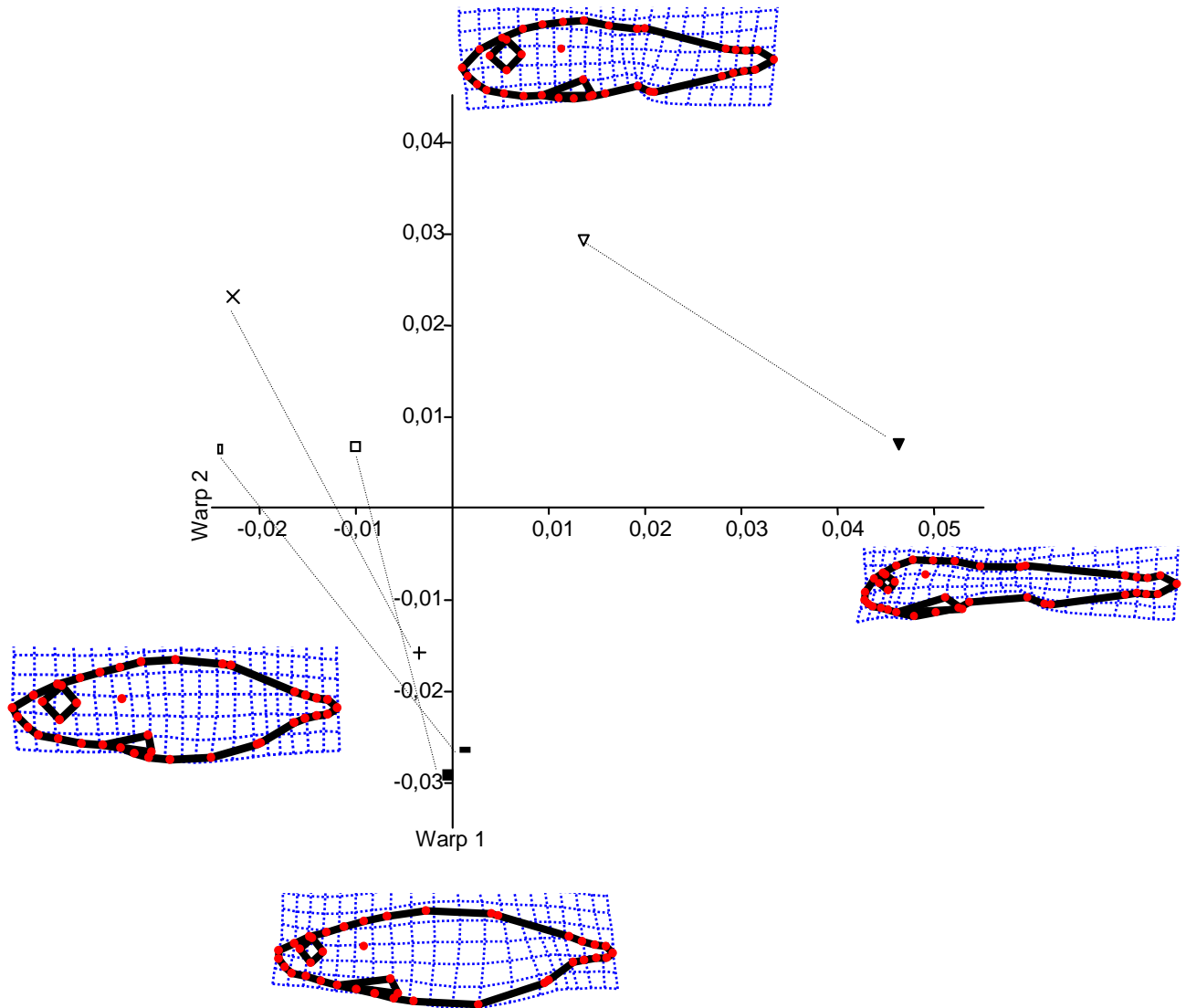


Fig. 9. First two relative warps (accounting for 81.7% of total shape variation) of *Gasterosteus aculeatus* sex-specific average shapes of the Adria grouped populations. Populations used are: the Neretva System (male: cross, female: plus), Isonzo River (male: empty bar, female: full bar,) Iznik Lake (male: empty square, female: full square) and Mulargia (male: empty inverse triangle, female: full inverse triangle). The means are calculated after a Procrustes fit of the landmark coordinations. Only adult ( $\geq 30$ mm SL) and not parasited specimens were used (n=194). Grey dotted lines connect the sexes of the same population. The deformation grids show deformations along the relative warps in relation to the overall consensus configuration (exaggerated by a factor of three).

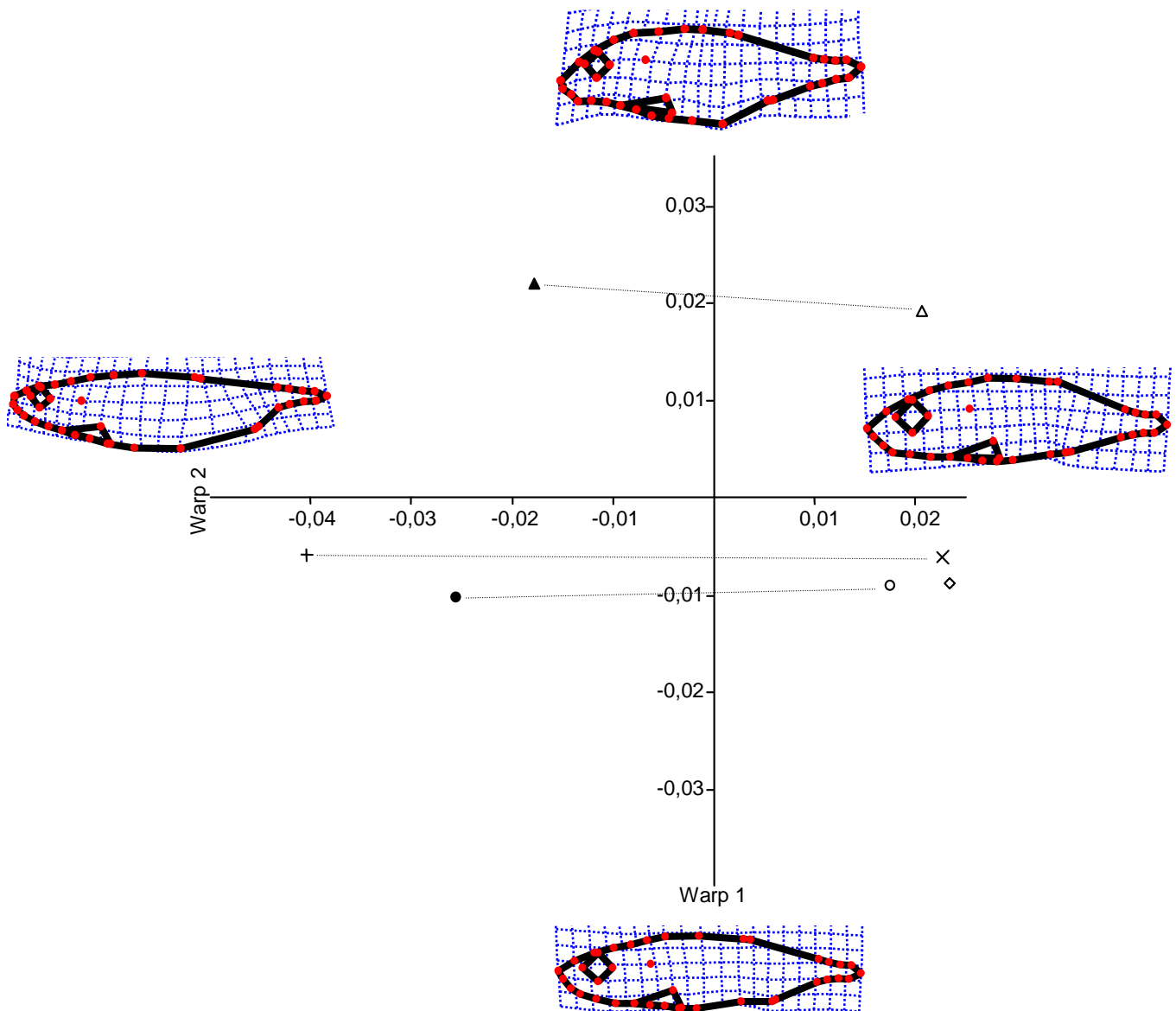


Fig. 10. First two relative warps (accounting for 86.9% of total shape variation) of *Gasterosteus aculeatus* sex-specific average shapes of the Neretva System populations, split by sex. Populations used are: Buna River (male: empty triangle, female: filled triangle), Hutovo Blato Wetland (male: empty diamond, no female specimens present), Neretva River (male: cross, female: plus), Norin River (male: empty circle, female: filled circle). The means are calculated after a Procrustes fit of the landmark coordinations. Only adult ( $\geq 30$ mm SL) and not parasited specimens were used. Grey dotted lines connect the sexes of the same population. The deformation grids show deformations along the relative warps in relation to the overall consensus configuration (exaggerated by a factor of three).

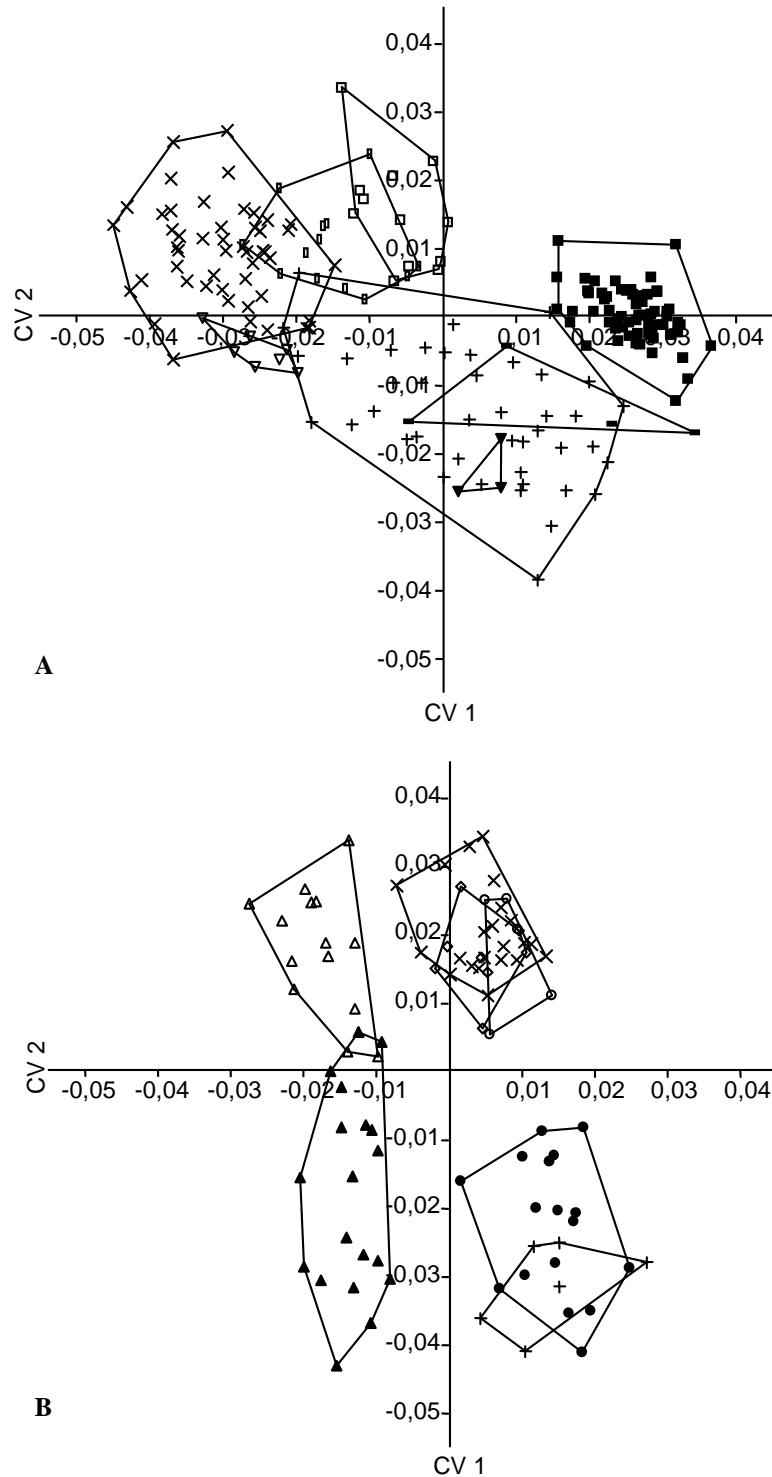


Fig. 11. Canonical variate analyses of the first 15 relative warps of *Gasterosteus aculeatus* landmark configurations. Only adult (SL  $\geq$  30mm) and not parasited specimens were used; A) CVA of Adria grouped populations, split by sex; Neretva System (male: cross, female: plus), Isonzo River (male: empty bar, female: filled minus) Iznik Lake (male: empty square, female: filled square), Mulargia (male: empty inverse triangle, female: filled inverse triangle) B) CVA of Neretva System populations, split by sex; Buna River (male: empty triangle, female filled triangle), Hutovo Blato Wetland (male: empty diamond, no female specimens present), Neretva River (male: cross, female plus ), Norin River (male: empty circle, female: filled circle).

A relative warp analysis of adult, not parasited specimens from all populations reveals that the first relative warp is mostly associated with body shape differences influenced by sex. These differences account for 39.3% of total shape variation. However, such differences may also be size dependent. To show these differences, the centroid size of the landmark configuration is used. A small centroid size indicates a small specimen, while a large centroid size indicates a large specimen. Shape differences between associated with centroid size are the head form, eye diameter, distances between the dorsal spines, anal fin length, length of ventral process and insertion point of the operculum membrane (Fig. 12F).

In all populations male specimens tend to have a lower average centroid size than female specimens (Fig. 12A-E). Male and female specimens from Buna River have similar centroid sizes, although females can reach larger centroid sizes than males. On the first relative warp there also exists a small overlapping area (Fig. 12A). Specimens from Neretva River were separated by sex on both traits, centroid size and first relative warp (Fig. 12B). This means, that female specimens were always larger than males and differed in body shape. Norin River specimens differed also on the first relative warp. Female sticklebacks, however, cover not only the same centroid size range as males, but can reach much larger centroid sizes than male sticklebacks (Fig. 12C). Isonzo River specimens did differ by sex on the first relative warp with only a small overlapping area. Female specimens had larger centroid sizes than males and overlap only in a small area (Fig. 12D). The reference populations show a bit different picture. While Iznik Lake specimens and Mulargia specimens separate into their sex clusters respectively on the first relative warp, they completely overlap regarding centroid size Fig. 12E). Only some female specimens from Iznik Lake can reach larger centroid sizes than males.

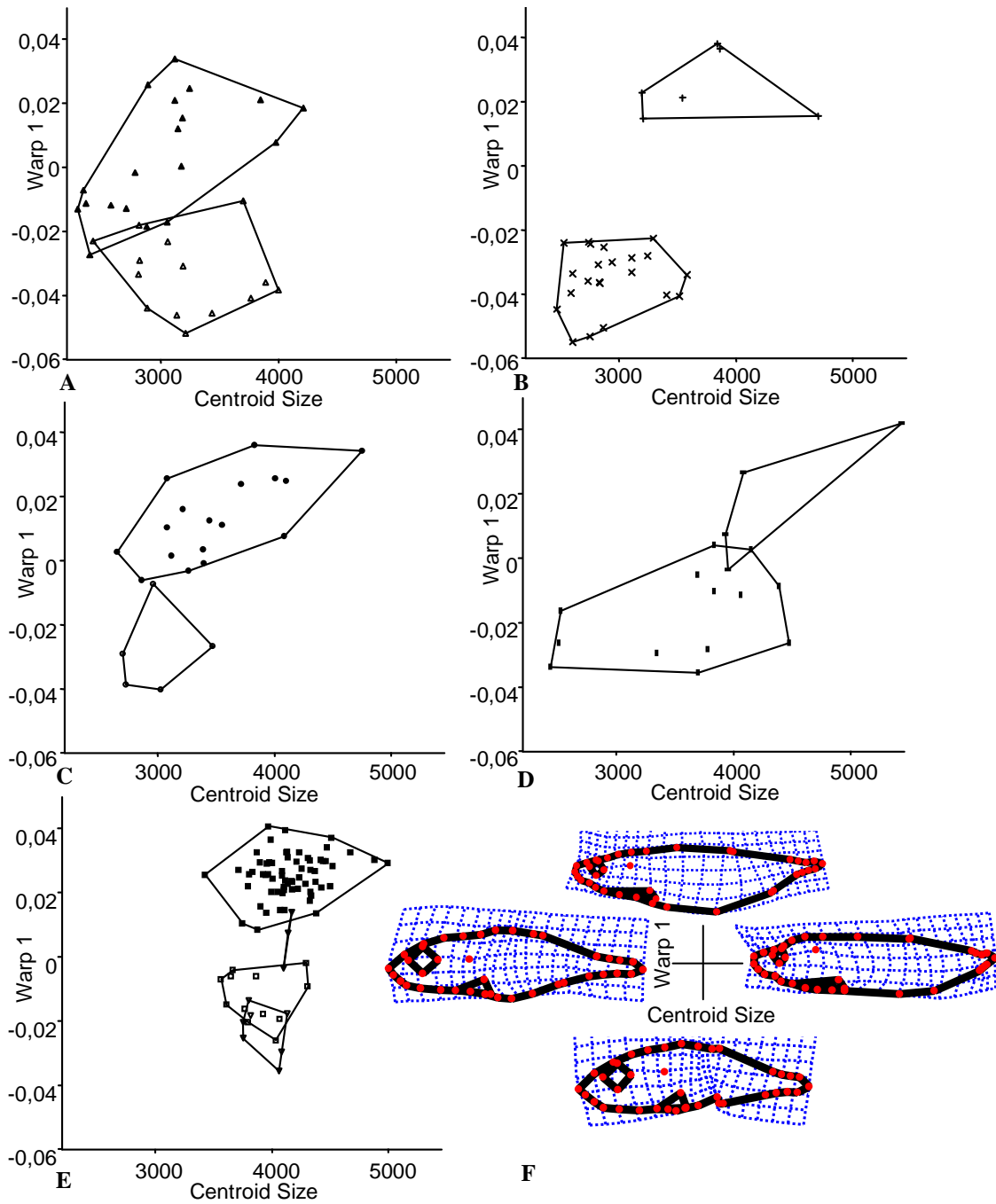


Fig. 12. Relation of *Gasterosteus aculeatus* centroid size with the first relative warp (accounting for 39.3% of total shape variation) with all populations together; only adult ( $\geq 30\text{mm}$ ) and not parasited specimens were used. All populations were both sexes are present are shown with split sexes. A) Bunia River (male: empty triangle, female: filled triangle). B) Neretva River (male: cross, female: plus). C) Norin River (male: empty circle, female: filled circle). D) Isonzo River (male: empty bar, female: filled minus). E) Reference populations Iznik Lake (male: empty square, female: filled square) and Mulargia (male: empty inverse triangle, female: filled inverse triangle). F) thin plate spline deformation grids. The deformation grids show deformations along the axes in relation to the overall consensus configuration (exaggerated by a factor of three).

A closer look at the Neretva System sticklebacks with another relative warp analysis confirms that the first relative warp shows sex differences. The first relative warp (accounting for 37.3% of total shape variation) shows similar differences as a relative warp computed without parasited specimens. It represent sex differences, mainly eye diameter, head form, distances between the dorsal spines, anal fin length, ventral process length, distance between anal spine and tip of ventral process and position of operculum membrane insertion point. The second relative warp (accounting for 15.1% of total shape variation) is mainly influenced by body shape differences caused by the parasite *Schistocephalus solidus*. This results in an expanded abdominal swelling, mainly anal fin length, distance between anal spine and tip of ventral process and ventral process length. Also a dorsal-ventral bending is observable (Fig. 13F). In Buna River only female specimens had positive scores for the first relative warp, while male specimens had low and negative scores (Fig. 13A). An overlapping area does exist with female specimens also having low negative scores. Specimens from Neretva River had the same tendency with positive scores on the first relative warp for female specimens and negative scores for male specimens. In this population no overlapping region exists on the first relative warp (Fig. 13B). Also mostly positive scores on the first relative warp are found for female sticklebacks in Norin River. Male specimens from Norin River had only negative scores. A large part of adult specimens from Norin River (45.7% in total; 37.8% of photographed specimens) were parasited by *Schistocephalus solidus*. This parasite can reach an enormous body length inside the body cavities of its host. This affects the body shape. *Gasterosteus aculeatus* with *S. solidus* in their bodies in Norin River often have a body shape similar to not parasited females, independent of their real sex (Fig. 13C). However the range of the parasited specimens on the first relative warp stretches from the average male to the average female specimens, blurring the borders of the two sexes set by the unparasited specimens. Specimens from Isonzo River show a clear sex separation along the first relative warp with no overlap. Female specimens are located in the positive range, male specimens in the negative (Fig. 13D). The reference populations, Iznik Lake and Mulargia, have the same tendency as the rest of the populations. On the first relative warp male specimens have negative scores, female specimens have positive scores. Only one parasited specimen exists in Mulargia. This specimen is male and shows no different body shape as other male Mulargia specimens (Fig. 13E).



Phenotypic diversity of *Gasterosteus aculeatus* in the Mediterranean Sea

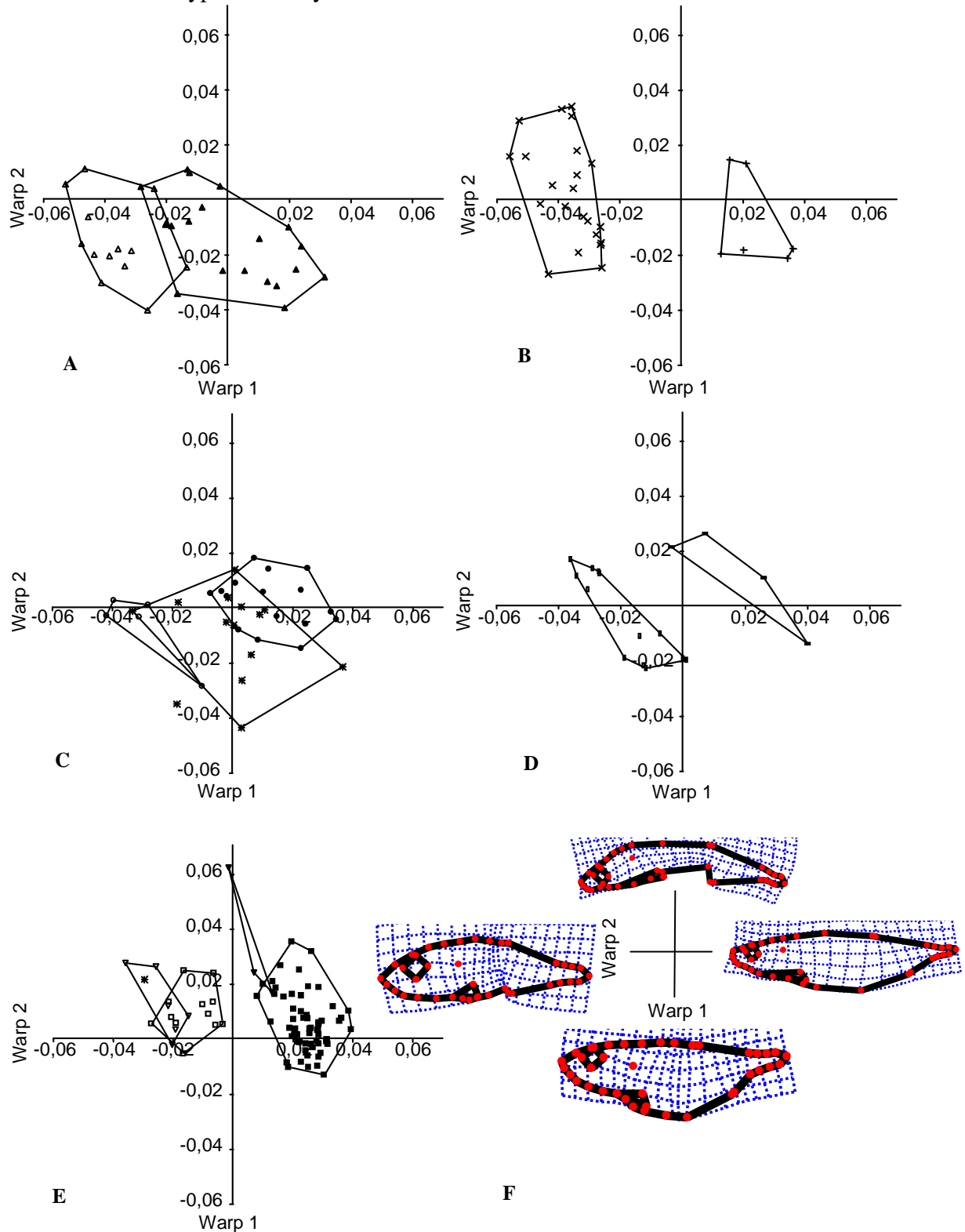


Fig. 13. First two relative warps (accounting for 52.4% of total shape variation) of *Gasterosteus aculeatus* with parasited specimens. Only adult (SL  $\geq$  30mm) specimens were used. All populations were both sexes are present are shown with split sexes. Parasited specimens are not split by sex and marked as a star. A) Buna River (male: empty triangle, female: filled triangle). B) Neretva River (male: cross, female: plus). C) Norin River (male: empty circle, female: filled circle). D) Isonzo River (male: empty bar, female: filled minus). E) Reference populations Iznik Lake (male: empty square, female: filled square) and Mulargia (male: empty inverse triangle, female: filled inverse triangle). F) thin plate spline deformation grids. The deformation grids show deformations along the axes in relation to the overall consensus configuration (exaggerated by a factor of three).

## DISTANCE RATIOS

Tab. 3. Mean values and ANOVA tests of the differences of distance ratios of Landmarks 5 to 6 and 3 to 4 of *Gasterosteus aculeatus* populations regarding sex. Pop=population; n=sampling size; mean=average mean value; STDV=standard deviation; var=variance; Lev.=p-value of Levene's-Test of Homogeneity of Variance; p=p-value of ANOVA.

Pop	Sex	n	Mean	STDV	Var	Lev.	p
Buna River	m	14	2.19	0.34	0.114	0.257	0.135
	f	19	2.04	0.23	0.051		
Neretva River	m	21	1.86	0.24	0.057	0.730	0.074
	f	6	1.66	0.20	0.041		
Norin River	m	5	1.78	0.22	0.048	0.348	0.178
	f	17	1.67	0.15	0.022		
Isoonzo River	m	12	1.66	0.19	0.038	0.705	0.916
	f	5	1.64	0.20	0.042		
Iznik Lake	m	12	1.60	0.17	0.027	0.684	0.001
	f	64	1.45	0.14	0.019		
Mulargia	m	7	2.27	0.26	0.066	0.389	0.128
	f	3	2.00	0.15	0.022		

Tab. 4. Mean values and ANOVA tests of the differences of distance ratios of Landmarks 5 to 6 and 2 to 4 of *Gasterosteus aculeatus* populations regarding sex. Pop=population; n=sampling size; mean=average mean value; STDV=standard deviation; var=variance; Lev.=p-value of Levene's-Test of Homogeneity of Variance; p=p-value of ANOVA.

Pop	Sex	n	Mean	STDV	Var	Lev.	p
Buna River	m	14	1.24	0.14	0.021	0.065	0.597
	f	19	1.22	0.08	0.007		
Neretva River	m	21	1.05	0.12	0.014	0.712	0.420
	f	6	1.01	0.11	0.011		
Norin River	m	5	1.04	0.07	0.004	0.932	0.110
	f	17	0.98	0.07	0.005		
Isoonzo River	m	12	0.98	0.10	0.009	0.383	0.857
	f	5	0.99	0.12	0.015		
Iznik Lake	m	12	0.91	0.07	0.005	0.710	0.023
	f	64	0.86	0.07	0.004		
Mulargia	m	7	1.30	0.09	0.008	0.138	0.060
	f	3	1.18	0.04	0.001		

Phenotypic diversity of *Gasterosteus aculeatus* in the Mediterranean Sea

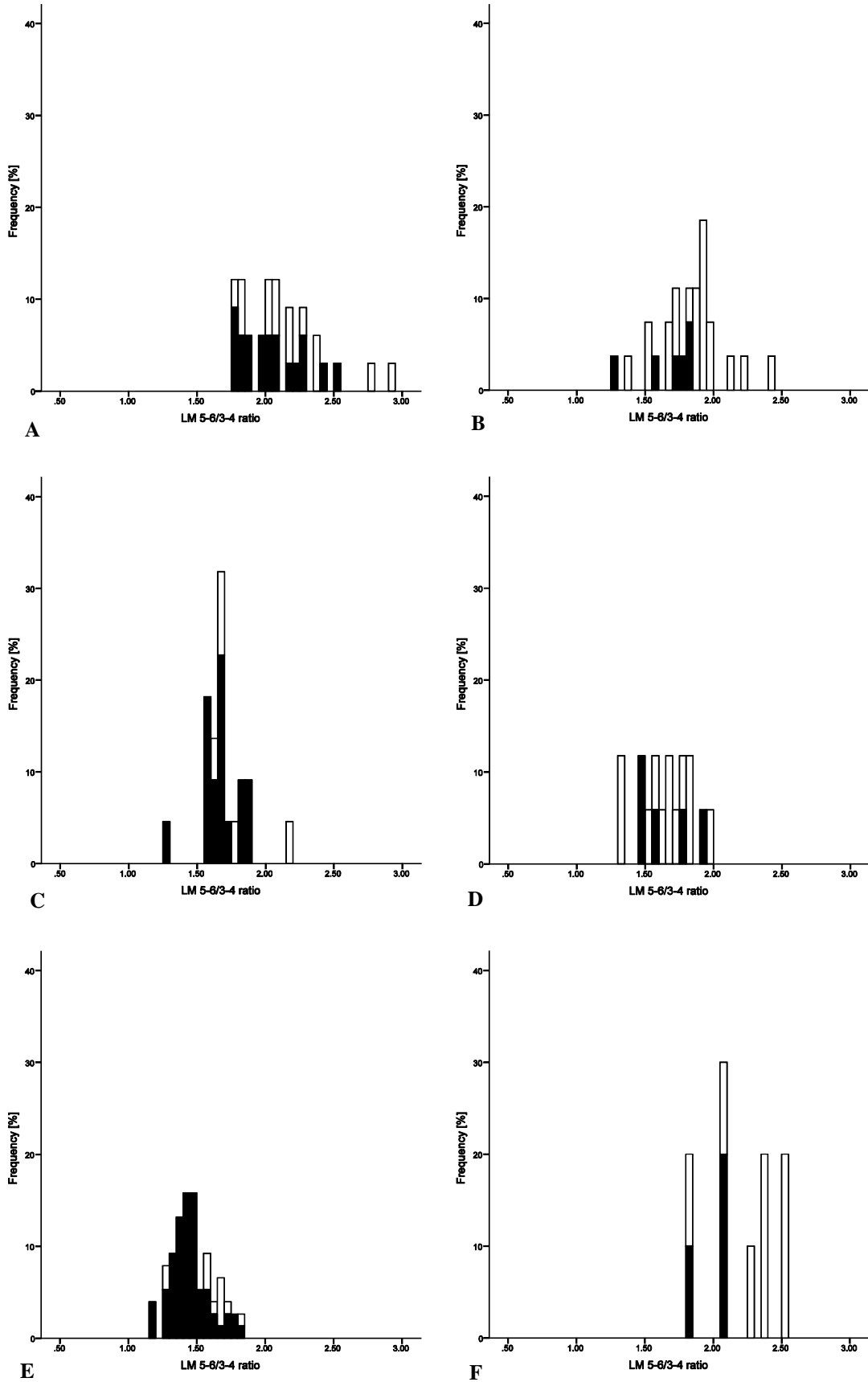


Fig. 14. Histograms of the ratio of the distances between the Landmarks 5 to 6 and 3 to 4 of *Gasterosteus aculeatus*. Shown are all sampling sites where sex separation was possible; male = white bars, female = black bars; A) Buna River, n=33; B) Neretva River, n=27; C) Norin River, n=22; D) Isonzo River, n=17; E) Iznik Lake, n=76; F) Mulargia, n=10.

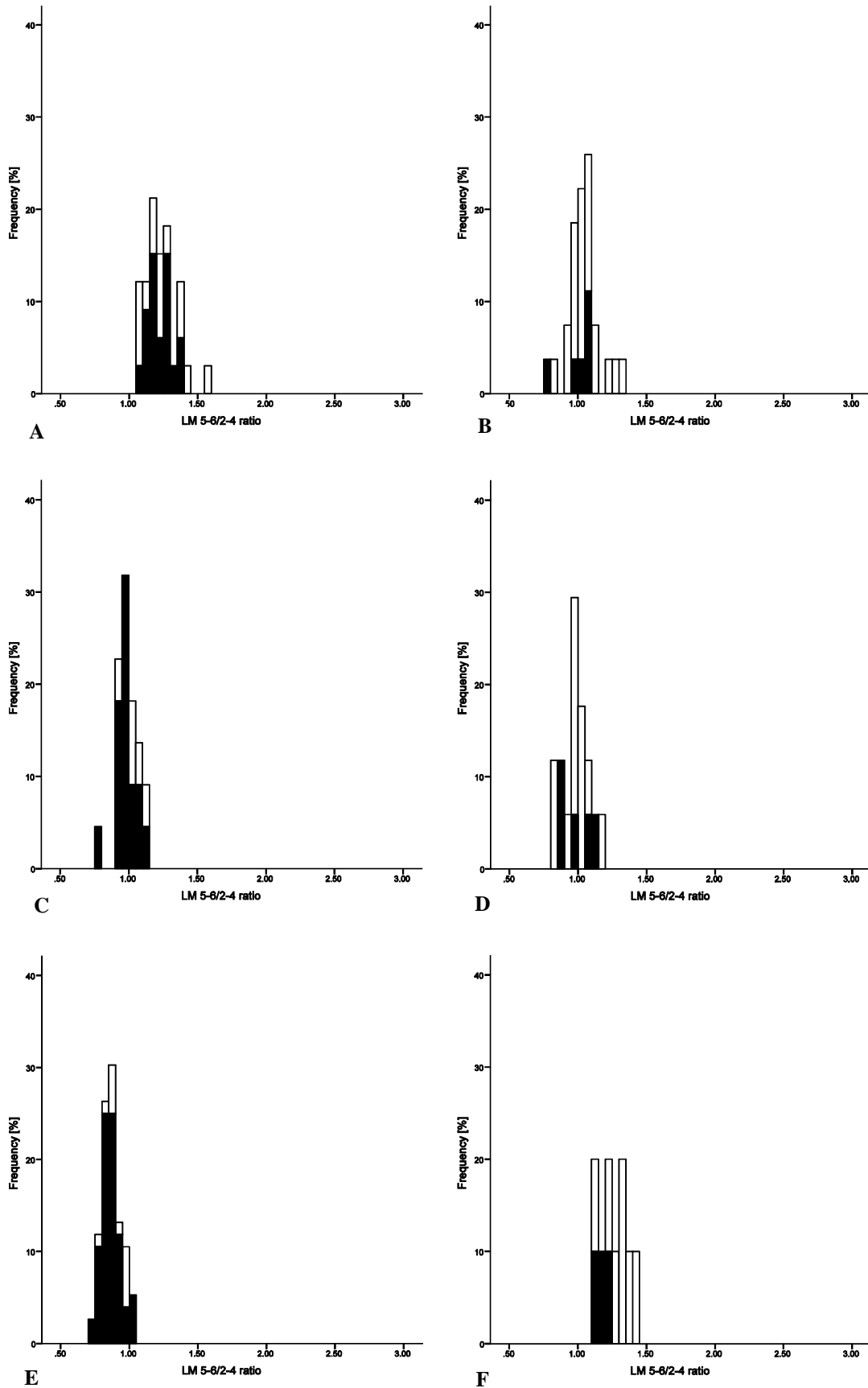


Fig. 15. Histograms of the ratio of the distances between the Landmarks 5 to 6 and 2 to 4 of *Gasterosteus aculeatus*. Shown are all sampling sites where sex separation was possible; male = white bars, female = black bars; A) Buna River, n=33; B) Neretva River, n=27; C) Norin River, n=22; D) Isonzo River, n=17; E) Iznik Lake, n=76; F) Mulargia, n=10.

One of the main differences between the sexes of *Gasterosteus aculeatus*, regardless of the observed population, is the distance between dorsal spines, especially from the second to the third dorsal spine (inter-dorsal-spine-distance, LM 3-4) (Fig. 2, Fig. 10, Fig. 12F, Fig. 13F). Female specimens seem to have a longer distance between the second and third dorsal spine while in male specimens the distance is shorter. The dorsal fin shows no difference between the sexes. Thus the ratio of the inter-dorsal spine distance to the dorsal fin length is expected to be different in male and female specimens. It is expected that females have a smaller ratio of these two distances than males. Histograms of the distribution of this ratio show, that female *G. aculeatus* have a lower ratio than males. However, there is no clear distinction between the two sexes. In Iznik Lake population (Fig. 14E) the distinction is the most visible, although also in this population the two sexes overlap. In the Neretva River populations (Fig. 14B) the least distinction between the sexes is possible.

A comparison of the mean ratios of the sexes within each population with ANOVAs revealed no significant differences except for Iznik Lake sticklebacks. All compared groups had similar variances (Levene test,  $p > 0.05$ ; Tab. 3). In Buna River sticklebacks the average female ratio is  $2.04 \pm 0.23$ , while the male ratio is slightly higher with  $2.19 \pm 0.34$  ( $p = 0.135$ ). Males also cover a larger range than females. The range of males, however, also covers the one of females, though it is only extended with higher ratios and not with lower ones (Fig. 14A). Male Neretva River specimens also have a higher average ratio ( $1.86 \pm 0.24$ ) than females ( $1.66 \pm 0.20$ ) ( $p = 0.074$ ). In this population the female range is also located within the male ones, although the female lowest value is found below the male one (Fig. 14B). In Norin River female sticklebacks have with  $1.67 \pm 0.15$  a lower average ratio than male sticklebacks ( $1.78 \pm 0.22$ ) ( $p = 0.178$ ). Male specimens are found within the range of the females, though the maximum values are found within the males (Fig. 14C). Male and female specimens from Isonzo River have nearly similar distributions (Fig. 14D). Also the mean values are nearly the same (males:  $1.66 \pm 0.19$ , females:  $1.64 \pm 0.20$ ,  $p = 0.916$ ). Thus no sex separation is visible in Isonzo River sticklebacks. Iznik Lake female sticklebacks have an average ratio of  $1.45 \pm 0.14$ . The ratio of male specimens is mostly located in the upper half of the range of females. The average ratio of male specimens is  $1.60 \pm 0.17$  ( $p \leq 0.001$ ). The lowest values are found in females (Fig. 14E). The female Mulargia sticklebacks show a ratio found in the lower half of the male ratio range (Fig. 14F). With an average ratio of  $2.00 \pm 0.15$  females have a lower ratio than males, which is  $2.27 \pm 0.26$  ( $p = 0.128$ ).

If the distance between the first dorsal spine and the third dorsal spine is used, instead of the distance between the second and third dorsal spine, the difference between the ratios of both sexes is even lower. Nearly no tendency can be observed. Both sexes share more or less the same ratios (Fig. 15A-F). Only in Mulargia male sticklebacks have a higher ratio than females (Tab. 4).

## DISCUSSION

In this study *Gasterosteus aculeatus* populations of the Adria drainage system have been analysed with geometric morphometric methods to compare body shapes from different freshwater systems. Body shapes from the Adria populations (Neretva System, Isonzo River) and reference populations (Iznik Lake, Mulargia) differ between the populations mainly by two factors: locality and sex. Specimens from Mulargia population show the most differences in body shape to all Adria populations. These differences are expressed in body height, head form and length of the dorsal and anal fin. Also the distance between the tip of the ventral process and the anal spine is different in Mulargia population.

Body shapes from within the Neretva System show also differences influenced mostly by two factors: also locality and sex. Specimens from Buna River are larger in body height, have smaller eyes and their dorsal spines are located closer together than elsewhere in the Neretva System. Also their ventral process as well as the anal fin is shorter than in the other Neretva System populations.

The second main difference found from both systems is the difference in body shape between male and female specimens. These differences are similar in all examined populations, not alone in the Adria System, but also include the reference populations. Male sticklebacks have a larger head and larger eyes than female sticklebacks. The distance between the tip of the ventral process and the anal spine is shorter in males than in females. Females have an increased abdominal area, which might be a result of gravidity of the female specimens. Also the distance between the first and last fin ray of the anal fin is shorter in females than in males.

Sexual dimorphism in *G. aculeatus* is a well-known fact and is shown in numerous studies (e.g. Sargent et al., 1984; Bakker & Mundwiler, 1999; Kitano, Mori & Peichel, 2007; Albert et al., 2008; Aguirre & Akinpelu, 2010; Kitano, Mori & Peichel, 2012). For instance male and female sticklebacks differ in size of the pectoral fin during breeding season with males having a larger pectoral fin than females (Bakker & Mundwiler, 1999). As a species with male parental care *G. aculeatus* males use the pectoral fin to oxygenate the eggs with fanning movements (Wootton, 1984; Bell & Foster, 1994a). Sexual dimorphism in body shape is similar around the globe for *G. aculeatus* populations. Aguirre & Akinpelu (2010) showed for several populations from Cook Inlet in Alaska, that male *G. aculeatus* have a larger head length than female *G. aculeatus*. Kitano, Mori & Peichel (2007) reported sexual dimorphism in traits like eye diameter and snout length

in populations from North America and Japan. These traits with sexual dimorphism were also observable in this study (see Results section). Kitano, Mori & Peichel (2007) as well as Aguirre et al. (2008) reported for female threespine sticklebacks a larger average standard length than males. This connection of sex with size was observable only to a small extent in some populations in this study by putting centroid size in relationship with the first relative warp of a relative warp analysis. The circumstance that in this study such a connection was mostly not visible might be explained through low sample sizes in some groups which probably lead to a biased sample. With larger sampling sizes it might be possible to observe a larger average female than male standard length.

Differences in body shape also exist depending on the habitat constraints (Reimchen et al., 1985; Spoljaric & Reimchen, 2007). Kitano, Mori & Peichel (2012) reported a reduction of sexual dimorphism in body depth in stream resident populations from North America and Japan compared to marine populations. A similar effect has already been found by Hagen & Gilberts (1972) for lake and stream populations. He reported that over distances of a few kilometres body depth and number of gill rakers can vary greatly and that they correlate with the habitat the population is located in. Webster et al. (2011) showed from the Great Eau drainage in Lincolnshire, eastern England, that, although the habitats are connected, differences in body shape between populations exist. These differences encompass eye diameter, body depth, shape of the caudal peduncle and relative spine sizes. However no sex has been determined. This might lead to difficulties in interpreting, since some aspects, like eye diameter but also body depth, as shown above, are known to be affected by sex.

Extensive genetic studies have been conducted to resolve the phylogeography and genetic relationships of *G. aculeatus* populations in Europe (Schluter et al., 2004; Cano et al., 2006; Mäkinen et al., 2006; Cano et al., 2008; Mäkinen & Merilä, 2008; Mäkinen et al., 2008; DeFaveri et al., 2012). DeFaveri et al. (2012) conducted extensive genetic comparisons of *G. aculeatus* populations in the drainage system of the Adriatic Sea and compared them with other populations in Europe, based upon data from Mäkinen, Cano & Merilä (2006) and Mäkinen & Merilä (2008). With mtDNA sequence data and mtDNA haplotypes DeFaveri et al. (2012) tried to resolve the phylogeography of *G. aculeatus* freshwater populations. Within the Neretva System they covered the rivers Buna, Bregava, Neretva and Norin. Based upon their mtDNA data, populations from the rivers Bregava, Neretva and Norin cluster close together and form a close relationship to each



other. Buna River population on the other hand forms a separate cluster and has a greater distance to the rest of the Neretva System populations. This results mirror the findings of this study, where body shape data results in a clustering of Hutovo Blato Wetland, Neretva River and Norin River populations. Buna River population clusters separately, the sticklebacks having a different average body shape than the rest of the Neretva System sticklebacks.

The populations of the Neretva System have always formed a separate group in recent genetic studies, or has been put together with other Mediterranean populations (Mäkinen et al., 2006; Cano et al., 2008; Mäkinen & Merilä, 2008). In haplotype networks or phylogenetic trees the Neretva System populations are found mostly next to Lake Skadar populations (Mäkinen et al., 2006; Mäkinen & Merilä, 2008; DeFaveri et al., 2012), while for instance Krka River populations are already counted to the European lineage like Mirna River populations (DeFaveri et al., 2012).

The driving forces behind phenotypic divergence between populations are mostly direct natural selection and genetic drift. In freshwater populations from North Europe Leinonen et al. (2006) showed that natural selection is the main factor for phenotypic divergence, although genetic drift cannot be dismissed. Since environmental factors like predators or amount of relative littoral area directly influence body shape of freshwater populations (Walker, 1997), such constraints can be selecting agents of stickleback populations in a freshwater system. If the ecological conditions within two freshwater systems are similar this would lead to a stabilising selection on body shape, resulting in similar body shapes (Kerschbaumer, Mitteröcker & Sturmbauer, 2012). Neutral markers on the other hand undergo genetic drift if the two freshwater systems are genetically isolated. Such a situation might be the case for populations in the Neretva System and Isonzo River. The two freshwater systems are isolated at least since the last glacial maximum (LGM) 18.000 years BP (Fairbanks, 1989; Peltier & Fairbanks, 2006) and if the ecological conditions, like predator regime or habitat diversifications, are similar, the average body shapes of the sticklebacks of both systems might be similar too, while neutral markers of mtDNA are not due to genetic drift. In the analysis of Mäkinen & Merilä (2008) populations from the Rivers Stella and Mirna have been used as the northern most populations of the Adriatic System. River Isonzo is located between them. Populations from River Stella and River Mirna cluster together in the European lineage of the phylogenetic tree of Mäkinen & Merilä (2008) while River Neretva populations form their own lineage. Also in the

phylogenetic tree of DeFavieri et al. (2012) populations from River Mirna are counted to the European lineage, while the populations from the Neretva System form the Adriatic lineage. This supports the view of this study that the longer isolation period of the Neretva System leads to different mtDNA while changes in phenotypes might not necessarily have occurred.

The opposite example seemed to have happened with the threespine sticklebacks of Mulargia. The body shape of Mulargia specimens shows differences mainly in body height and head shape. Specimens are compressed and have short heads. Such a different body shape compared to other populations suggests different ecological constraints. Further Mulargia specimens showed an extreme reduced number of lateral plates, up to the point of zero on both sides, as well as reduced lengths of dorsal and ventral spines (not reported in this study). Such reduced elements of the defensive complex are typical for Systems with a lack of piscivore fishes (Gross, 1978; Reimchen, 1983; Reimchen, 1994), but might also have an influence on the body shape. Gross (1978) and Reimchen (1983) showed that the elements of the defensive apparatus (defensive complex) encapsulate the anterior part of the body. If the lateral plates in the defensive complex are completely absent the capsule in the anterior part of the body does not stabilize the body and a compressed form might be possible. On the other hand the compressed body shape might be a fixation artefact, because of the dehydrating effect of alcohol.

The phenotype of a threespine stickleback might also be influenced by assortative mating. Snowberg & Bolnick (2012) showed in two populations of Burnt-Out Lake in British Columbia that in the presence of phenotypic divergence threespine sticklebacks show signs of mate preferences based upon diet. Furin, von Hippel & Bell (2012) reviewed that assortative mating often is based upon body size and thus can lead by standing genetic variation to reproductive isolation between the primitiv anadromous population and a newly founded freshwater population. Thus, by the combination of assortative mating and standing genetic variation, a different body shape in a newly found population can arise within a very short time period.

Schluter & Conte (2009) explained the presence of morphological differences between marine and freshwater sticklebacks, by repeated colonization of freshwater habitats through anadrome sticklebacks. They formulated the transporter hypothesis, which gives an idea how freshwater populations can evolve rapidly and show distinguishable different

morphotypes by having standing genetic variation in marine populations which derives to high frequencies in newly colonized freshwater habitats.

Although the relative warp analyses of the sex-specific average shapes of *G. aculeatus* revealed certain groupings of the populations, such groupings were not that obvious if all specimens were used as single cases in such analyses (data not shown in this study). Therefore canonical variate analyses were used to achieve a better group separation. The CVA actually failed to do this, although some trends were observable. Within the male and female sticklebacks respectively the populations show similar distributions and large overlapping areas. Only sex seems to contribute enough differences in body shape to have an effect on group separation. Other factors which are based upon the populations alone weigh not enough to separate the populations in a CVA.

The only population that shows differences in the CVA is the Buna River population. This population groups separately to all other Neretva River populations, males and females respectively. As already mentioned above body shape differs in Buna River sticklebacks from all other populations. Already Vuković & Kosorić (1967) showed that Buna River specimens have different morphological traits than other stickleback populations within the Neretva System. Vuković & Kosorić (1967) compared the relative length of the first and second dorsal spine of Buna river specimens with the specimens from Norin River examined by Karaman (1928) and found relative shorter spines in Buna River specimens than the spines found in Norin River specimens. DeFaveri et al. (2012) also separate Buna River populations from the rest of the Neretva River populations, especially populations from Norin River and Neretva River, based upon mtDNA sequence data and mtDNA haplotypes and thus mirrors the findings based upon geometric morphometric data revealed by this study. As already mentioned above, within a freshwater system populations can differ in their phenotypic expressions over the distance of a few kilometres even if they are physically connected (Hagen & Gilberts, 1972; Webster et al., 2011).

The thin plate spline deformation grids of the relative warp analyses revealed differences between male and female sticklebacks in body shape, as discussed above. One of the differences was different distances between the dorsal spines. Male had dorsal spines located closer together than females. Since in males and females the length of the dorsal fin was more or less similar a distance ratio of the inter-dorsal-spine distance and the

dorsal fin length might be different in male and female sticklebacks. This was not the fact, since ANOVA tests failed to show convincing significant differences in the inter-dorsal-spine distance of male and female sticklebacks, regardless of the population. This leads to the conclusion that the difference of male and female body shapes is comprised of many different aspects and traits. The distance between dorsal spines is only one among many (e.g. Kitano et al., 2007; Aguirre et al., 2008; Aguirre & Akinpelu, 2010) and the classification of a specimen as male or female cannot be done by a single ratio of two distances. More than one external trait is needed to be included, although sex determination only by external morphological might yield in false classifications. It is better to use gonad inspection or genetic analyses, than relying on external morphological features for sex determination.

The threespine stickleback *G. aculeatus* is the only fish host recognized for the plerocercoids of the pseudophyllidean cestod *Schistocephalus solidus* and get infected when they feed on infected copepods and are thus the second host in the three-host life cycle of *S. solidus* (Barber & Scharsack, 2010). The plerocercoid can reach enormous body lengths relative to the length of *G. aculeatus* and alters not only phenotypic traits but also swimming and risk-taking behaviour, body conditions and gonad development (reviewed in Barber & Scharsack, 2010).

Norin River population was the only population of the examined populations with a large percentage (45.7% of the total sample) of specimens being infected with the parasite *S. solidus*. The infected specimens were all female and the body shape of infected specimens showed an intermediate form of not infected male and female specimens. The parasite *S. solidus* is known to be able to reach enormous lengths of several times the body length of its host (Barber & Scharsack, 2010). The longer the parasite is the more space in the body cavity is needed and the result is a swelling of the lower body, similar to the swelling of the abdominal region of a gravid female. Thus infected *G. aculeatus* can have a body shape similar to gravid females. If the parasite does not take up such a large space in the body cavity the body shape is similar to not gravid specimens. Female specimens who are parasite by *S. solidus* have a disadvantage in maturation of the eggs and show reduced gonad development (reviewed in Barber & Scharsack, 2010). The presence of *S. solidus* influences the development of *G. aculeatus* (Barber & Scharsack, 2010) and is possible the reason that infected female specimens of Norin River population show an intermediate form between not infected male and female threespine sticklebacks.

## CONCLUSIONS

The populations of the threespine stickleback, *Gasterosteus aculeatus*, of the Adria Drainage System differ in body shape not so much between the populations but rather within the Systems themselves. Although the freshwater systems of the Rivers Isonzo and Neretva have been separated for thousands of years (Fairbanks, 1989; Peltier & Fairbanks, 2006) the morphological differences are rather small. The Neretva System itself houses different populations that partially show distinguishable phenotypes. Sticklebacks from Buna River, as the most stream upwards population, show morphological differences not found in the other examined populations of the Neretva System. This is possibly because of habitat constraints like environmental conditions or predator pressure (e.g. Hagen & Gilberts, 1972; Gross, 1978; Reimchen, 1994; Webster et al., 2011).

The most striking phenotypic variation universal in all examined populations are body shape differences between male and female threespine stickleback. As found in populations in Europe, America and Japan (Kitano et al., 2007; Aguirre & Akinpelu, 2010; Webster et al., 2011), sexual dimorphism in head morphology and other traits along the body are also found in the populations of the Adria Drainage System. Thus the traits subjected to sexual dimorphism seem to be universal all over the globe.

Specimens of the threespine stickleback infected with the parasite *Schistocephalus solidus* exhibit body shapes between not infected male and female specimens. Thus a specimen with a swelled abdomen is not necessarily a gravid female but could also be a heavily parasited specimen. Also a specimen with no abdominal swelling can house *S. solidus* when the parasite is small and does not cover much space in the body cavity.

Although traits like head size, eye diameter, distances between the dorsal spines differ between the sexes, sex determination cannot be done by external observation or by measured distances, as was shown with the ratio of two distances in this study. Although *G. aculeatus* males exhibits breeding colouration during the breeding season (Wootton, 1984), this as well as body shape, is not a reliable information if regarded alone. Sex should best be determined by gonad inspection or genetic analyses. The differences between two populations, however, is best analysed with combined techniques. The stickleback populations from Isonzo River and the Neretva System show differences in neutral genetic markers as revealed by DeFavieri et al. (2012), but the average body shapes exhibit only marginal differences. Thus using both analyses is in accordance.

## REFERENCES

- Aguirre WE. 2009.** Microgeographical diversification of threespine stickleback: body shape–habitat correlations in a small, ecologically diverse Alaskan drainage. *Biological Journal of the Linnean Society* **98**: 139-151.
- Aguirre WE, Akinpelu O. 2010.** Sexual dimorphism of head morphology in three-spined stickleback *Gasterosteus aculeatus*. *Journal of Fish Biology* **77**: 802-821.
- Aguirre WE, Ellis KE, Kusenda M, Bell MA. 2008.** Phenotypic variation and sexual dimorphism in anadromous threespine stickleback: implications for postglacial adaptive radiation. *Biological Journal of the Linnean Society* **95**: 465-478.
- Ahn DG, Gibson G. 1999.** Axial variation in the threespine stickleback: genetic and environmental factors. *Evolution & Development* **1**: 100-112.
- Ahnelt H. 2011.** Two new sympatric *Knipowitschia* species (Teleostei: Gobiidae) from an eastern Mediterranean coastal lake - examples of different dispersal patterns? *Zootaxa* **3114**: 22-30.
- Ahnelt H, Bianco PG, Schwammer H. 1995.** Systematics and zoogeography of *Knipowitschia caucasica* (Teleostei: Gobiidae) based on new records from the Aegean Anatolian area. *Ichthyological Exploration of Freshwaters* **6**: 49-60.
- Ahnelt H, Muerth P, Lunardon A. 2006a.** Erster Nachweis einer vierten Lateralplattform des Dreistachligen Stichlings *Gasterosteus aculeatus* (Teleostei, Gasterosteidae) in Österreich. *Österreichs Fischerei* **59**: 156-159.
- Ahnelt H, Pohl H, Hilgers H, Splechtna H. 1998.** The threespine stickleback in Austria (*Gasterosteus aculeatus* L., Pisces: Gasterosteidae) - Morphological variations. *Annalen des Naturhistorischen Museums Wien* **100 B**: 395-404.
- Ahnelt H, Pohl H, Miljković N, Hilgers H. 2006b.** Phenotypic diversity in the threespine stickleback *Gasterosteus aculeatus* Linnaeus, 1758 (Teleostei: Gasterosteidae) in western Austria - the four-spined form. *Annalen des Naturhistorischen Museums Wien* **107 B**: 25-38.
- Albert AYK, Sawaya S, Vines TH, Knecht AK, Miller CT, Summers BR, Balabhadra S, Kingsley DM, Schluter D. 2008.** The genetics of adaptive shape shift in stickleback: Pleiotropy and effect size. *Evolution* **62**: 76-85.
- Bakker TCM, Mundwiler B. 1999.** Pectoral fin size in a fish species with paternal care: a condition-dependent sexual trait revealing infection status. *Freshwater Biology* **41**: 543-551.
- Banbura J, Bakker TCM. 1995.** Lateral plate morph genetics revisited: Evidence for a fourth morph in three-spined sticklebacks. *Behaviour* **132**: 1153-1171.
- Barber I, Scharsack JP. 2010.** The three-spined stickleback-*Schistocephalus solidus* system: an experimental model for investigating host-parasite interactions in fish. *Parasitology* **137**: 411-424.
- Barrett RDH. 2010.** Adaptive evolution of lateral plates in three-spined stickleback *Gasterosteus aculeatus*: a case study in functional analysis of natural variation. *Journal of Fish Biology* **77**: 311-328.
- Baumgartner JV. 1992.** Spatial variation of morphology in a freshwater population of the threespine stickleback, *Gasterosteus aculeatus*. *Canadian Journal of Zoology* **70**: 1140-1148.
- Bell MA. 1981.** Lateral plate polymorphism and ontogeny of the complete plate morph of threespine sticklebacks (*Gasterosteus aculeatus*). *Evolution* **35**: 67-74.
- Bell MA, Andrews CA. 1997.** Evolutionary consequences of postglacial colonization of fresh water by primitively anadromous fishes. In: Streit B, Städler T and Lively

- CM, eds. *Evolutionary Ecology of Freshwater Animals: Concepts and Case Studies*. Basel: Birkhäuser Verlag. 323-363.
- Bell MA, Foster SA. 1994a.** *The Evolutionary Biology of the Threespine Stickleback*. Oxford University Press: Oxford.
- Bell MA, Foster SA. 1994b.** Introduction to the evolutionary biology of the threespine stickleback. In: Bell MA and Foster SA, eds. *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press. 1-27.
- Bianco PG. 1980.** Areale Italic, rinvenimento in Calabria e origini delle popolazioni mediterranee di *Gasterosteus aculeatus* L. (Pisces, Gasterosteidae). *Bollettino del Museo civico di storia naturale di Verona* **7**: 197-216.
- Bianco PG, Ahnelt H, Economidis PS. 1996.** The freshwater fishes from eastern, and large Mediterranean islands with comments on their safety status. *Acta Universitatis Carolinae Biologica* **40**: 45-60.
- Bianco PG, Miller PJ. 1990.** Yugoslavian and other records of the Italian freshwater goby, *Padogobius martensii*, and a character polarization in gobioid fishes. *Journal of Natural History* **24**: 1289-1302.
- Bookstein FL. 1991.** *Morphometric Tools for Landmark Data: Geometry and Biology*. Cambridge University Press: Cambridge.
- Caldecutt WJ, Adams DC. 1998.** Morphometrics of trophic osteology in the threespine stickleback, *Gasterosteus aculeatus*. *Copeia*: 827-838.
- Campbell RN. 1985.** Morphological variation in the three-spined stickleback (*Gasterosteus aculeatus*) in Scotland. *Behaviour* **93**: 161-168.
- Cano JM, Mäkinen HS, Leinonen T, Freyhof J, Merilä J. 2008.** Extreme neutral genetic and morphological divergence supports classification of Adriatic three-spined stickleback (*Gasterosteus aculeatus*) populations as distinct conservation units. *Biological Conservation* **141**: 1055-1066.
- Cano JM, Matsuba C, Mäkinen H, Merilä J. 2006.** The utility of QTL-Linked markers to detect selective sweeps in natural populations - a case study of the EDA gene and a linked marker in threespine stickleback. *Molecular Ecology* **15**: 4613-4621.
- Cavalcanti MJ. 2008.** nts2csv, format conversion program. Universidade do Estado do Rio de Janeiro, Brasil: Departamento de Zoologia.
- Clavero M, Pou-Rovira Q, Zamora L. 2009.** Biology and habitat use of three-spined stickleback (*Gasterosteus aculeatus*) in intermittent Mediterranean streams. *Ecology of Freshwater Fish* **18**: 550-559.
- Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G, Dickson M, Grimwood J, Schmutz J, Myers RM, Schluter D, Kingsley DM. 2005.** Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science* **307**: 1928-1933.
- Correggiari A, Roveri M, Trincardi F. 1996.** Late Pleistocene and Holocene evolution of the northern Adriatic sea. *Il Quaternario Italian Journal of Quaternary Sciences* **9**: 697-704.
- Crivelli AJ, Britton RH. 1987.** Life history adaptations of *Gasterosteus aculeatus* in a Mediterranean wetland. *Environmental Biology of Fishes* **18**: 109-125.
- DeFaveri J, Zanella LN, Zanella D, Mrakovčić M, Merilä J. 2012.** Phylogeography of isolated freshwater three-spined stickleback *Gasterosteus aculeatus* populations in the Adriatic Sea basin. *Journal of Fish Biology* **80**: 61-85.
- Fairbanks RG. 1989.** A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. *Nature* **342**: 637-642.

- Filipe AF, Araújo MB, Doadrio I, Angermeier PL, Collares-Pereira MJ. 2009.** Biogeography of Iberian freshwater fishes revisited: the roles of historical versus contemporary constraints. *Journal of Biogeography* **36**: 2096-2110.
- Foster SA. 1994.** Evolution of the reproductive behaviour of threespine stickleback. In: Bell MA and Foster SA, eds. *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press. 381-398.
- Furin CG, von Hippel FA, Bell MA. 2012.** Partial reproductive isolation of a recently derived resident-freshwater population of threespine stickleback (*Gasterosteus aculeatus*) from its putative anadromous ancestor. *Evolution*: in press.
- Giles N. 1983.** The possible role of environmental calcium levels during the evolution of phenotypic diversity in Outer Hebridean populations of the Three-spined stickleback, *Gasterosteus aculeatus*. *Journal of Zoology* **199**: 535-544.
- Gross HP. 1978.** Natural selection by predators on the defensive apparatus of the three spined stickleback, *Gasterosteus aculeatus* L. *Canadian Journal of Zoology* **56**: 398-413.
- Hagen DW, Gilberts LG. 1972.** Geographic variation and environmental selection in *Gasterosteus aculeatus* L. in the Pacific Northwest, America. *Evolution* **26**: 32-51.
- Hammer Ø, Harper DAT, Ryan PD. 2001.** PAST: Palaeontological Statistics software package for education and data analysis. *Palaeontologia Electronica* **4**: 9.
- Hermida M, Fernandez J, Amaro R, San Miguel E. 2005.** Morphometric and meristic variation in Galician threespine stickleback populations, northwest Spain. *Environmental Biology of Fishes* **73**: 189-200.
- Hopkins K, Moss BR, Gill AB. 2011.** Increased ambient temperature alters the parental care behaviour and reproductive success of the three-spined stickleback (*Gasterosteus aculeatus*). *Environmental Biology of Fishes* **90**: 121-129.
- Karaman S. 1928.** Прилози Ихтиологији Југославије I. (Beiträge zur Ichthyologie von Jugoslavien I). *Bulletin de la Société Scientifique de Skoplje* **6**: 147-176.
- Kawahara R, Miya M, Mabuchi K, Near TJ, Nishida M. 2009.** Stickleback phylogenies resolved: Evidence from mitochondrial genomes and 11 nuclear genes. *Molecular Phylogenetics and Evolution* **50**: 401-404.
- Kerschbaumer M, Mitteröcker P, Sturmbauer C. 2012.** Pattern of body shape change in sympatric versus non-sympatric *Tropheus* populations of Lake Tanganyika. *Heredity*: unpublished.
- Kitano J, Bolnick DI, Beauchamp DA, Mazur MM, Mori S, Nakano T, Peichel CL. 2008.** Reverse evolution of armor plates in the threespine stickleback. *Current biology* **18**: 769-774.
- Kitano J, Mori S, Peichel CL. 2007.** Sexual dimorphism in the external morphology of the threespine stickleback (*Gasterosteus aculeatus*). *Copeia*: 336-349.
- Kitano J, Mori S, Peichel CL. 2012.** Reduction of sexual dimorphism in stream-resident forms of three-spined stickleback *Gasterosteus aculeatus*. *Journal of Fish Biology* **80**: 131-146.
- Kleinlercher G, Muerth P, Pohl H, Ahnelt H. 2008.** Welche Stichlingsart kommt in Österreich vor, *Gasterosteus aculeatus* oder *Gasterosteus gymnurus*? *Österreichs Fischerei* **61**: 158-161.
- Klepaker T. 1995.** Postglacial evolution in lateral plate morphs in Norwegian freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). *Canadian Journal of Zoology* **73**: 898-906.
- Kotellat M. 1997.** European freshwater fishes. *Biologia, Bratislava - Section Zoology* **52 (Supplement 5)**: 1-271.



- Leinonen T, Cano JM, Mäkinen HS, Merilä J. 2006.** Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology* **19**: 1803-1812.
- Lobon-Cervia J, Penczak T, de Sostoa A. 1988.** Morphological variability and distribution of stickleback (*Gasterosteus aculeatus* L.) in Spain. *Cybiurn* **12**: 219-227.
- Maceda-Veiga A, Monleon-Getino A, Caiola N, Casals F, de Sostoa A. 2010.** Changes in fish assemblages in catchments in north-eastern Spain: biodiversity, conservation status and introduced species. *Freshwater Biology* **55**: 1734-1746.
- Magalhães MF, Beja P, Schlosser IJ, Collares-Pereira MJ. 2007.** Effects of multi-year droughts on fish assemblages of seasonally drying Mediterranean streams. *Freshwater Biology* **52**: 1494-1510.
- Mäkinen HS, Cano JM, Merilä J. 2006.** Genetic relationships among marine and freshwater populations of the European three-spined stickleback (*Gasterosteus aculeatus*) revealed by microsatellites. *Molecular Ecology* **15**: 1519-1534.
- Mäkinen HS, Merilä J. 2008.** Mitochondrial DNA phylogeography of the three-spined stickleback (*Gasterosteus aculeatus*) in Europe - Evidence for multiple glacial refugia. *Molecular Phylogenetics and Evolution* **46**: 167-182.
- Mäkinen HS, Shikano T, Cano JM, Merilä J. 2008.** Hitchhiking mapping reveals a candidate genomic region for natural selection in three-spined stickleback chromosome VIII. *Genetics* **178**: 453-465.
- Marchinko KB, Schluter D. 2007.** Parallel evolution by correlated response: Lateral plate reduction in threespine stickleback. *Evolution* **61**: 1084-1090.
- McKinnon JS, Rundle HD. 2002.** Speciation in nature: the threespine stickleback model systems. *Trends in Ecology & Evolution* **17**: 480-488.
- Mitteroecker P, Gunz P. 2009.** Advances in Geometric Morphometrics. *Evolutionary Biology* **36**: 235-247.
- Morrell LJ, Hentley WT, Wickens VJ, Wickens JB, Rodgers GM. 2012.** Artificial enhancement of an extended phenotype signal increases investment in courtship by three-spined sticklebacks. *Animal Behaviour* **84**: 93-101.
- Mrakovcic M, Misetic S, Povz M. 1995.** Status of freshwater fish in Croatian Adriatic river systems. *Biological Conservation* **72**: 179-185.
- Münzing J. 1962.** Ein neuer *semiarmatus*-Typ von *Gasterosteus aculeatus* L. (Pisces) aus dem Izniksee. *Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut* **60**: 181-194.
- Münzing J. 1963.** Evolution of variation and distributional patterns in European populations of the three-spined stickleback, *Gasterosteus aculeatus*. *Evolution* **17**: 320-332.
- Nelson JS. 2006.** *Fishes of the world*. John Wiley & Sons: Hoboken, New Jersey.
- Özuluğ M, Altun Ö, Meriç N. 2005.** On the fish fauna of Lake Iznik (Turkey). *Turkish Journal of Zoology* **29**: 371-375.
- Paepke H-J. 2002.** *Gasterosteus* Linnaeus, 1758. In: Bănărescu PM and Paepke H-J, eds. *The Freshwater Fishes of Europe*. Wiebelsheim: AULA-Verlag GmbH. 206-256.
- Peltier WR, Fairbanks RG. 2006.** Global glacial ice volume and Last Glacial Maximum duration from an extended Barbados sea level record. *Quaternary Science Reviews* **25**: 3322-3337.
- Penczak T. 1965.** Morphological variation of the stickleback (*Gasterosteus aculeatus* L.) in Poland. *Zoologica Poloniae* **15**: 3-49.
- Rabineau M, Berné S, Olivet J-L, Aslanian D, Guillocheau F, Joseph P. 2006.** Paleo sea levels reconsidered from direct observation of paleoshoreline position during

- Glacial Maxima (for the last 500,000 yr). *Earth and Planetary Science Letters* **252**: 119-137.
- Reimchen TE. 1983.** Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). *Evolution* **37**: 913-946.
- Reimchen TE. 1992.** Injuries on stickleback from attacks by a toothed predator (*Oncorhynchus*) and implications for the evolution of lateral plates. *Evolution* **46**: 1224-1230.
- Reimchen TE. 1994.** Predators and morphological evolution in threespine stickleback. In: Bell MA and Foster SA, eds. *The Evolutionary Biology of the Threespine Stickleback*. Oxford: Oxford University Press. 241-276.
- Reimchen TE, Stinson EM, Nelson JS. 1985.** Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. *Canadian Journal of Zoology* **63**: 2944-2951.
- Rohlf FJ. 2004.** tpsSpline, thin-plate spline. 1.20 ed. State University of New York at Stony Brook: Department of Ecology and Evolution.
- Rohlf FJ. 2008a.** tpsDig, digitize landmarks and outlines. 2.12 ed. State University of New York at Stony Brook: Department of Ecology and Evolution.
- Rohlf FJ. 2008b.** tpsRelw, relative warps analysis. 1.46 ed. State University of New York at Stony Brook: Department of Ecology and Evolution.
- Rohlf FJ. 2009a.** tpsRegr, shape regression. 1.37 ed. State University of New York at Stony Brook: Department of Ecology and Evolution.
- Rohlf FJ. 2009b.** tpsUtil, file utility program. 1.44 ed. State University of New York at Stony Brook: Department of Ecology and Evolution.
- Sánchez-Hernández J, Vieira-Lanero R, Servia MJ, Cobo F. 2011.** Feeding habits of four sympatric fish species in the Iberian Peninsula: keys to understanding coexistence using prey traits. *Hydrobiologia* **667**: 119-132.
- Sargent RC, Bell MA, Krueger WH, Baumgartner JV. 1984.** A lateral plate cline, sexual dimorphism, and phenotypic variation in the black-spotted stickleback, *Gasterosteus wheatlandi*. *Canadian Journal of Zoology* **62**: 368-376.
- Schluter D, Clifford EA, Nemethy M, McKinnon JS. 2004.** Parallel evolution and inheritance of quantitative traits. *American Naturalist* **163**: 809-822.
- Schluter D, Conte GL. 2009.** Genetics and ecological speciation. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 9955-9962.
- Sharpe DMT, Räsänen K, Berner D, Hendry AP. 2008.** Genetic and environmental contributions to the morphology of lake and stream stickleback: implications for gene flow and reproductive isolation. *Evolutionary Ecology Research* **10**: 849-866.
- Snowberg LK, Bolnick DI. 2012.** Partitioning the effects of spatial isolation, nest habitat, and individual diet in causing assortative mating within a population of threespine stickleback. *Evolution*: in press.
- Spoljaric MA, Reimchen TE. 2007.** 10 000 years later: evolution of body shape in Haida Gwaii three-spined stickleback. *Journal of Fish Biology* **70**: 1484-1503.
- Tarkan AS, Gaygusuz Ö, Acipinar H, Gürsoy Ç, Özuluğ M. 2006.** Length-weight relationship of fishes from the Marmara region (NW-Turkey). *Journal of Applied Ichthyology* **22**: 271-273.
- Taylor EB, McPhail JD. 1986.** Prolonged and burst swimming in anadromous and freshwater threespine stickleback, *Gasterosteus aculeatus*. *Canadian Journal of Zoology* **64**: 416-420.
- van Straaten LMJU. 1971.** Holocene and late-Pleistocene sedimentation in the Adriatic Sea. *Geologische Rundschau* **60**: 106-131.

- Vila-Gispert A, Alcaraz C, García-Berthou E. 2005.** Life-history traits of invasive fish in small Mediterranean streams. In: Capdevila-Argüelles L and Zilletti B, eds. *Issues in Bioinvasion Science, EEI 2003: a Contribution to the Knowledge on Invasive Alien Species*. Dordrecht: Springer. 107-116.
- Vizi A, Vizi O. 2010.** Changes in the diet composition of Pygmy Cormorant *Phalacrocorax pygmeus* in Skadar Lake (Southern Montenegro). *Acrocephalus* **31**: 21-26.
- Vuković T, Kosorić Đ. 1967.** Uzrasno variranje dužine dorzalnih bodlji kod *Gasterosteus aculeatus* Linne (koljuška, bodonja) iz reke Bune. *Ribarstvo Jugoslavije* **12**: 7-9.
- Vuković T, Prolić F. 1966.** Prilog poznavanju taksonomskih karakteristika *Gasterosteus aculeatus* Linné iz donje Neretve. *Glasnik zemaljskog muzeja, Prirodne nauke* **5**: 175-178.
- Walker JA. 1997.** Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L (Gasterosteidae) body shape. *Biological Journal of the Linnean Society* **61**: 3-50.
- Webster MM, Atton N, Hart PJB, Ward AJW. 2011.** Habitat-specific morphological variation among threespine sticklebacks (*Gasterosteus aculeatus*) within a drainage basin. *PLoS ONE* **6**: e21060.
- Wooton RJ. 1984.** *A Functional Biology of Sticklebacks*. Croom Helm: London & Sydney.
- Zanella LN. 2009.** Varijabilnost i morfološke značajke koljuške *Gasterosteus aculeatus* (Linnaeus, 1758) u Hrvatskoj. Magistra prirodnih znanosti biologije, Magistarski rad, Sveučilište u Zagrebu.
- Zanella LN, Zanella D, Mrakovcic M, Miletic M, Mustafic P, Caleta M. 2009.** Occurrence of four-spined *Gasterosteus aculeatus* in an isolated Croatian river population. *Journal of Fish Biology* **75**: 2052-2061.
- Ziuganov VV. 1983.** Genetics of osteal plate polymorphism and microevolution of threespine stickleback (*Gasterosteus aculeatus* L.). *Theoretical and Applied Genetics* **65**: 239-246.

## ACKNOWLEDGEMENTS

My thanks go to many people, first of all to Univ. Doz. Dr. Harald Ahnelt. He supposed this Master thesis and for this I thank him as well as for his endless and patient supervision. I also thank Univ. Ass. Dr. Philipp Mitteröcker for further supervision and especially for the statistical education he granted me. My thanks also go to Univ. Prof. Dr. Dr. Gerd Müller, the head of the Department of Theoretical Biology, University of Vienna, for making this study possible at all.


I also thank PD Dr. Ralf Thiel, Head of Section Ichthyology, Biocenter Grindel and Zoological Museum, University of Hamburg for the loan of the specimens from the Iznik Lake, Turkey and from Sardinia, Italy. These thanks also go to Dr. Ernst Mikschi, Head of the Vertebrate Department, Curator of the Ichthyological Collection, Naturhistorisches Museum Wien for the loan of the specimens from the Isonzo River, Italy.

Further acknowledgements go to the members of the Institute of Oceanography and Fisheries in Split, Croatia. Here I thank Prof. dr. sc Jakov Dulčić, without whom the work at the Institute would not have been possible. Also I thank Dr. sc Pero Tutmann who supported me every day in every possible way at the Institute. I would have no material to work on, if not for him. Also I thank all the colleagues at the Institute in Split for comments and insights during my stay in Split. I exhibit further thanks for the financial support from the Institute of Oceanography and Fisheries in Split for my stays in Split. Many thanks go to my wife, Mag.<sup>a</sup> Anna Maria Rokop, who's never faltering belief in me nourished and supported me in times of doubt of myself and when I did not believe to ever finish this thesis.

Further thanks got to all colleagues at the Department of Theoretical Biology at the University of Vienna for their comments, support and discussions.

This study was partially financed by the short-term grant abroad (KWA) commissioned by Research Services and International Relations, University of Vienna, Austria.

## CURRICULUM VITAE

<b>Personal information</b>  	Name:	Markus ROKOP, BSc
	Date of birth:	15 February 1981
	Place of birth:	Vienna
	Nationality:	Austria
	University Address:	Department of Theoretical Biology, University Vienna Althanstraße 14 1090 Vienna
	Private Address:	Mittelgasse 11/32 1060 Vienna
	email:	a0000118@unet.univie.ac.at
Phone:	+436643652292	
<b>Education</b>	since Nov. 2008	Master Study Zoology at the <b>University of Vienna</b>
	Nov. 2008	Bachelor of Science Degree in Biology; <b>Faculty of Life Sciences, University of Vienna</b>
	Oct. 2000 - Nov. 2008	Diploma-Studies of Biology at the <b>University of Vienna</b>
	1991 - June 2000	Grammar School: <b>BG/BRG 5, Rainergasse, Vienna</b>
	1987 - 1991	Elementary School in <b>Vienna</b>
<b>Bachelor degree</b>	BSc-Thesis	Phaenotypic diversity of the threespine stickleback <i>Gasterosteus aculeatus</i> LINNAEUS, 1758 (Teleostei: Gasterosteidae) - a comparison of Populations from West- and East- Austria

---

<b>Job experience</b>	since Aug. 2011	Zookeeper for seawater at <b>Haus des Meeres, Vienna</b>
	2003 - 2011	Security Services for Jewish Museum and Kunsthistorisches Museum in <b>Vienna</b>
	Feb. 2002 - Jan. 2003	Civilian Service at Lebenshilfe in <b>Vienna</b>
	July 2001	Office work for <b>Siemens AG</b>

---

<b>Research at foreign institutes</b>	Aug. - Sep. 2009	Institute of Oceanography and Fisheries in <b>Split, Croatia</b>
	May 2010	

---

<b>Practical experience</b>	Comparative Anatomy and Morphology of fishes in <b>Sylt, Germany</b>	
	Fishes: functional and comparative anatomy	
	Anatomy of native fishes	
	Submicroscopical Anatomy & Practical Electron Microscopy	
	Microscopic anatomy of vertebrates	
	Sea Turtle Project - Protection of sea turtles in <b>Yaniklar, Turkey</b>	
	Advanced Marine Ecology at Marine biological Station STARESO in <b>Calvi, Korsika, France</b>	
	Fauna and Flora of marine habitats in <b>Rovinj, Croatia</b>	
	Animal observations in the zoo, <b>Tiergarten Schönbrunn, Vienna</b>	
	Applied Statistics for Biologists	
	Multivariate Statistics	
	<b>July 2012</b>	Workshop “Exploration of the underwater research lab”, together with Mag. phil. Cyril Dworsky, for the <b>Children’s University 2012, Vienna</b>
	<b>July 2010</b>	Workshop “Adventure under water”, together with Mag. phil. Cyril Dworsky, for the <b>Children’s University 2010, Vienna</b>
	<b>Summer Term 2011</b>	Tutor for the course: Tools in Cognitive Science II: Basic Statistics for Cognitive Scientists at <b>University of Vienna</b>
	<b>Summer Term 2012</b>	

---

---

<b>Languages</b>	German (Mother tongue) English
------------------	-----------------------------------

---

<b>IT Skills</b>	MS Office (Word, Excel, Access, Power Point) SPSS PAST tps Program Series End Note Graphic Programs (Paint Shop Pro)
------------------	---

---