

Microhabitat niche partitioning in sympatric species of genus *Gouania* Nardo, 1833 (Actinopterygii: Gobiesocidae) in the Northern Adriatic

Pamić, Maja

Master's thesis / Diplomski rad

2021

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Zagreb, Faculty of Science / Sveučilište u Zagrebu, Prirodoslovno-matematički fakultet**

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:217:454445>

Rights / Prava: [In copyright](#)/[Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2024-11-21**



Repository / Repozitorij:

[Repository of the Faculty of Science - University of Zagreb](#)



University of Zagreb
Faculty of Science
Department of Biology

Maja Pamić

**Microhabitat niche partitioning in sympatric species
of genus *Gouania* Nardo, 1833 (Actinopterygii:
Gobiesocidae) in the Northern Adriatic**

Master thesis



Zagreb, 2021

Sveučilište u Zagrebu
Prirodoslovno-matematički fakultet
Biološki odsjek

Maja Pamić

**Diferencijacija niša mikrostaništa simpatrijskih
vrsta roda *Gouania* Nardo, 1833 (Actinopterygii:
Gobiesocidae) sjevernog Jadrana**

Diplomski rad

Zagreb, 2021.

Ovaj rad je izrađen na Zoologijskom zavodu Biološkog odsjeka Prirodoslovno-matematičkog fakulteta u Zagrebu, pod voditeljstvom doc. dr. sc. Zorana Marčića. Rad je predan na ocjenu Biološkom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu radi stjecanja zvanja magistra struke znanosti o okolišu.

Acknowledgements

Many thanks to doc. dr. sc. Zoran Marčić for his guidance, encouragement and introduction to M. Sc Maximilian Wagner. I want to thank M. Sc Maximilian Wagner for his patience, mentorship and great support throughout the making of this thesis. I am grateful that they introduced me to the beautiful world of cryptobenthic fishes and gave me the opportunity to write such an interesting thesis. Lastly, thanks to doc. dr. sc. Kristina Pikelj for her advice and help with the geological part of the thesis.

TEMELJNA DOKUMENTACIJSKA KARTICA

Sveučilište u Zagrebu
Prirodoslovno-matematički fakultet
Biološki odsjek

Diplomski rad

Diferencijacija niša mikrostaništa simpatrijskih vrsta roda *Gouania*

Nardo, 1833 (Actinopterygii: Gobiesocidae) sjevernog Jadrana

Maja Pamić

Rooseveltov trg 6, 10000 Zagreb, Hrvatska

Rod *Gouania* endemičan je za Sredozemno more i predstavnik je rijetkih vrsta europskih kralješnjaka koji isključivo nastanjuju šljunkovitu obalu. Do 2019. godine smatralo se da je ovaj rod riba monotipski, ali nedavna filogenetska i taksonomska istraživanja dokazuju postojanje pet vrsta, od kojih dvije naseljavaju infralitoralni šljunak sjevernog Jadrana. Dosadašnja istraživanja bila su vezana za vrstu *Gouania willdenowi*, a njezinom redeskripcijom te pronalaskom novih vrsta javila se potreba za novim ekološkim istraživanjima. *Gouania pigra* i *Gouania adriatica* su simpatrijske vrste koje nastanjuju intersticijski prostor šljunkovitih plaža sjevernog Jadrana, a cilj ovoga istraživanja je bio istražiti diferencijaciju niša njihovog mikrostaništa. U ovom istraživanju uzorkovane su plaže u mjestu Sveta Marina, uvali Krnička luka i u Puli u kojima je prethodno utvrđena prisutnost ciljanih vrsta. Nasumično je kantom uzorkovan šljunak i među šljunkom su tražene jedinke roda *Gouania*. Utvrđene jedinke roda *Gouania* determinirane su i izmjerene, dok je šljunak prosijavan kroz niz sita. Statističkom obradom i analizom podataka istražene su razlike u mikrostaništu ovih dviju vrsta. Ustanovljeno je preklapanje niša vrsta *G. adriatica* i *G. pigra*, ali s povećanjem veličine tijela utvrđena je diferencijacija u najvećim frakcijama sedimenta. Osim veličine tijela, utvrđeno je da nekoliko granulometrijskih parametara utječu na odabir odgovarajućeg mikrostaništa. *Gouania pigra* je endem Jadrana i bolje poznavanje njezinog staništa, koji je pod iznimnim antropogenim pritiskom, pomoći će u njegovoj adekvatnoj zaštiti.

(40 stranica, 19 slika, 4 tablica, 40 literaturnih navoda, jezik izvornika: engleski)

Rad je pohranjen u Središnjoj biološkoj knjižnici

Ključne riječi: kriptobentičke ribe, infralitoral, šljunčana plaža, endem, priljepnjaci

Voditelj: doc. dr. sc. Zoran Marčić

Ocjenitelji:

doc. dr. sc. Zoran Marčić

doc. dr. sc. Kristina Pikelj

prof. dr. sc. Sven Jelaska

prof. dr. sc. Nenad Buzjak

Rad prihvaćen: 17.02. 2021.

BASIC DOCUMENTATION CARD

University of Zagreb
Faculty of Science
Department of Biology

Master Thesis

Microhabitat niche partitioning in sympatric species of genus *Gouania* Nardo, 1833 (Actinopterygii: Gobiesocidae) in the Northern Adriatic

Maja Pamić

Rooseveltova trg 6, 10000 Zagreb, Hrvatska

The genus *Gouania* is endemic to the Mediterranean Sea and is a representative of rare European vertebrate fauna which exclusively inhabits pebble beaches. Until 2019, this fish genus was considered monotypic, but recent phylogenetic and taxonomic research proved the existence of five species, two of which inhabit the infralittoral gravel of the northern Adriatic. Previous research has been related to the species *Gouania willdenowi*, but due to its redescription and description of the new species the need for new ecological research arose. *Gouania pigra* and *Gouania adriatica* are sympatric species which inhabit the interstitial space of pebble beaches of the northern Adriatic and the aim of this study was to investigate the differentiation of niches of their microhabitat. In this research, the beaches at Sveta Marina, Krnička Luka bay and in Pula, where the presence of both species was previously determined, were sampled. Gravel was sampled at random with a bucket and individuals of the genus *Gouania* were sought among the gravel. Identified individuals of the genus *Gouania* were determined and measured, and gravel was sifted through a series of sieves. Statistical processing and analysis of the data has determined the investigated differences in the microhabitat of these two species. An overlap in the niches of *G. adriatica* and *G. pigra* was found, but with an increase in body size, partitioning was established in the largest sediment fractions. Along with the body size, several granulometric parameters were found to influence the selection of the suitable microhabitat. *Gouania pigra* is endemic to the Adriatic and a better knowledge of its habitat, which is under extreme anthropogenic pressure, will help in its adequate protection.

(40 pages, 19 figures, 4 tables, 40 references, original in: English)

Thesis is deposited in Central Biological Library.

Keywords: cryptobenthic fishes, infralittoral, pebble beach, endemic, clingfish

Supervisor: doc. dr. sc. Zoran Marčić

Reviewers:

doc. dr. sc. Zoran Marčić

doc. dr. sc. Kristina Pikelj

prof. dr. sc. Sven Jelaska

prof. dr. sc. Nenad Buzjak

Thesis accepted: 17.02.2021.

CONTENTS

1.	INTRODUCTION.....	1
1.1.	Cryptobenthic fishes and the genus <i>Goaunia</i> Nardo, 1833.....	1
1.1.1.	<i>Gouania pigra</i> (Nardo, 1827).....	2
1.1.2.	<i>Gouania adriatica</i> Wagner, Kovačić and Koblmüller, 2020.....	4
1.2.	Microhabitat niche partitioning.....	5
1.3.	Aim of this thesis.....	6
2.	RESEARCH AREA.....	7
3.	MATERIALS AND METHODS.....	12
3.1.	Sampling.....	12
3.2.	Granulometrics.....	14
4.	RESULTS.....	14
4.1.	Sampling and body length of species.....	17
4.2.	Relative mass comparison.....	19
4.3.	Sieve analysis.....	24
5.	DISCUSSION.....	30
5.1.	Microhabitat niche partitioning in <i>Gouania</i>	30
5.2.	Geology as a proxy for species occurrence?.....	33
5.3.	Anthropogenic habitat alterations and species threats.....	34
6.	CONCLUSION.....	35
7.	LITERATURE.....	36
8.	CURRICULUM VITAE.....	38

1. INTRODUCTION

1.1. Cryptobenthic fishes and the genus *Gouania* Nardo, 1833

Cryptobenthic fishes are broadly defined by Depczynski and Bellwood (2003) as “adult fishes, typically smaller than 5 cm total length that are visually and/or behaviourally cryptic, and maintain a close association with the benthos” (see also Brandl et al. 2018). Additionally, Kovačić et al. (2012) provides a more specific definition of cryptobenthic fishes as “a fish species or life history stages of fish species that exclusively or predominantly spend their lifetime in cryptobenthic microhabitats, that is, in the restricted living spaces underneath the bottom surface of the substrate or biocover, with a physical barrier to the open spaces”. In general, cryptobenthic fishes are considered as a poorly studied group of fishes whose diversity is underestimated, primarily due to the lack of taxonomic expertise and efficient sampling methods (Brandl et al. 2018).

One family of cryptobenthic fishes occurring in the Mediterranean and the Adriatic Sea are the clingfishes (Gobiesocidae). Mediterranean clingfishes are characterised by a small size and a cryptobenthic lifestyle, which provides a good protection against predators (Hofrichter and Patzner 2000). Above all, clingfish have a thoracic disc, that allows adhesion to irregular surfaces in intertidal habitat and enables them to persist in a high energetic environment (Hofrichter and Patzner 2000; Wainwright et al. 2013).

Among over 35.700 valid fish species (Fricke et al. 2020a), only the Mediterranean clingfish genus *Gouania* Nardo, 1833 (Gobiesocidae) and Pacific gobies (Gobiidae) of the genus *Luciogobius* Gill, 1859 inhabit the interstitial space of intertidal gravel beaches (Yamada et al. 2009; Wagner et al. 2019). Genus *Gouania* is a Mediterranean endemic genus whose individuals inhabit exclusively the intertidal environment of gravel and boulder beaches (Wagner et al. 2020). A taxonomic classification of the genus is shown in **Table 1**. Until 2020, *Gouania* was composed of only one species, *Gouania willdenowi* (Risso, 1810), but recent work by Wagner et al. (2019, 2020) has shown existence of at least five different species. With the redescription of the species *G. willdenowi* (Wagner et al. 2020), its distribution range has changed and is now limited to the western part of the Mediterranean Sea exclusively. Accordingly, the findings in the Adriatic Sea refer to the species *G. pigra* (Nardo, 1827) and *G. adriatica* Wagner, Kovačić and Koblmüller, 2020. Above all, in areas

of sympatric species occurrences, *Gouania* species coexist in two very distinct morphotypes “slender” and “stout” that evolved independently in the Adriatic Sea and the oriental Mediterranean basin (Wagner et al. 2019, 2020). Despite overall body shape differences, the slender morph is characterized by an increased number of vertebrae and smaller eyes compared to its sympatric stout congener (Wagner et al. 2019, 2020).

Table 1. Classification of genus *Gouania* by Fricke et al. (2020a, 2020b)

Systematic category	Name
Kingdom	Animalia
Phylum	Chordata
Subphylum	Vertebrata
Superclass	Gnathostomata
Class	Actinopterygii
Order	Gobiesociformes
Family	Gobiesocidae
Subfamily	Lepadogastrinae
Genus	<i>Gouania</i>

1.1.1. *Gouania pigra* (Nardo, 1827)

Gouania pigra, commonly named as the “piglet sucker”, is considered as the only strictly marine endemic fish species in the Adriatic Sea (Wagner et al. 2020). It is synonymous with *Lepadogaster piger* Nardo, 1827, *Gouania prototypus* Nardo, 1833, *Gouania piger* Bonaparte, 1846 and *Leptopterygius piger* Günther, 1861, but due to lacking holotypes, the species was redescribed in 2020 (Wagner et al. 2020). Its geographic distribution ranges from northern Adriatic to Albany, with the southernmost record in Vlorë (Wagner et al. 2020). Throughout its range, this species is found in sympatry with *G. adriatica*. In Pelješac *G. pigra* can be found, beside *G. adriatica*, with the other “slender” *Gouania* morphotype, *G. hofrichteri* Wagner, Kovačić and Koblmüller, 2020 (**Figure 1**). *G. pigra* is typically abundant in fine gravel of intertidal pebble beaches and during low tide, it can be found in exposed area

(Hofrichter and Patzner 2000; Wagner et al. 2020). Besides their habitat preferences, little is known about the ecology and biology of the species and quantitative data is scarce (Wagner et al. 2020).

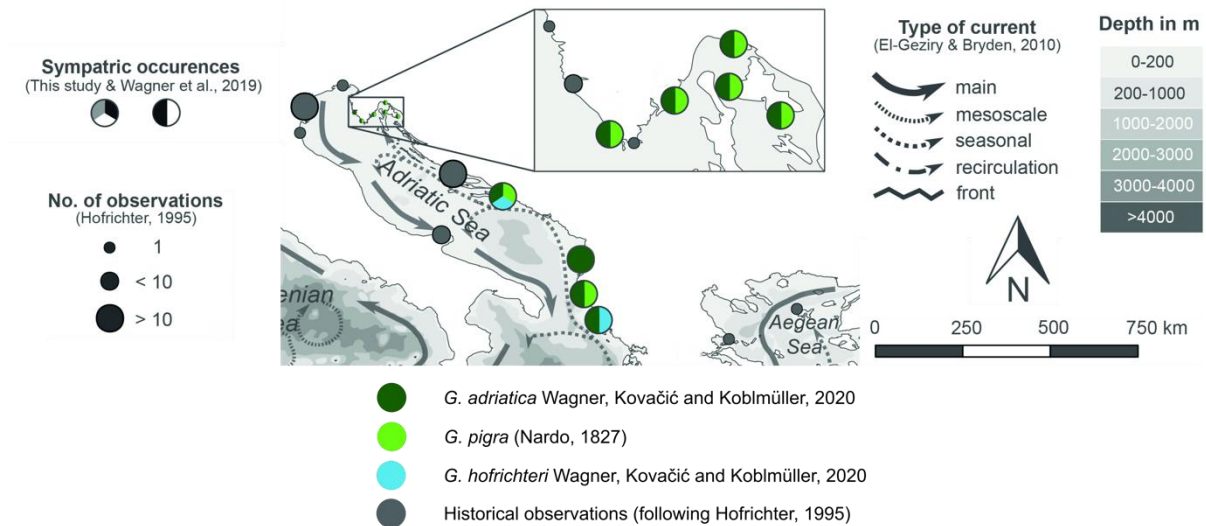


Figure 1. Geographical distribution ranges and sympatric occurrences. Figure changed after Wagner et al. (2020).

The piglet sucker is very slender and elongated, whereas the body is posteriorly and laterally compressed while the head is small and dorsoventrally compressed (Wagner et al. 2020). It can reach a maximum length of about 40 mm (Wagner et al. 2020). *G. pigra* is white to flesh-coloured, almost pigmentless or with a poorly visible irregular marbled pattern (**Figure 2**). Compared to stout morphotypes of *Gouania*, no star shaped pigmentation around the eyes is visible (Wagner et al. 2020). The slender-bodied *G. pigra* differs from all other congeners by its reduced pigmentation and the position of the posterior angle of jaws. Furthermore, *G. pigra* differs from stout-bodied species of *Gouania* by the dorsal head profile which is "S" curved, the absence of star-like pigmentation around eyes and the number of vertebrae (35-38). It is easily distinguishable from the sympatric *G. adriatica* by the posterior opercular edge, which has two clear tips (the upper longer or equal to the lower), the number of caudal rays (10-11 vs. 12-13) as well as vertebrae and the above-mentioned absence of star-pigmentation around the eyes (Wagner et al. 2020).



Figure 2. *Gouania pigra* (Nardo, 1827), taken from Wagner et al. (2020). Photos by M. Wagner and M. Kovačić.

1.1.2. *Gouania adriatica* Wagner, Kovačić and Koblmüller, 2020

Gouania adriatica, also known as the “Adriatic blunt-snouted clingfish”, is one of three stout-bodied species of *Gouania*. It inhabits intertidal pebble beaches throughout the Adriatic Sea and the northern Ionian Sea (**Figure 1**). The species occurs in sympatry with *G. pigra* throughout its distribution and at Pelješac, like the above-mentioned *G. pigra*, also in syntopy with *G. hofrichteri* (Wagner et al. 2020). *G. adriatica* is also found above the waterline during extremely low tide, mostly in late winter and early spring. There is almost no knowledge of ecology, biology and behaviour of the species, as it was the case with *G. pigra* (Wagner et al. 2020).

The body of *G. adriatica* is elongated, slender and posteriorly laterally compressed with a length varying from 20 mm to about 42 mm and a large head (Wagner et al. 2020). The snout is wide and blunt, as the common name suggests. *G. adriatica* is yellow to flesh-coloured, sometimes with irregular melanocytes decreasing towards the posterior part of the body and a star-like pigmentation around the eyes (**Figure 3**) (Wagner et al. 2020). Adriatic

blunt-snouted clingfish differs from slender-bodied species of *Gouania* by a low number of the vertebrae (35), a dorsal head profile that forms a straight line between nape above the eye and the upper lip tip and the previously mentioned star-like pigmentation. Additionally, *G. adriatica* differs from the sympatric congeneric, *G. pigra*, by the position of the posterior jaws angle as well as the number of principal caudal-fin rays (12-13 vs. 10-11). The species differs from other stout-bodied species by reduced pigmentation and by a posterior opercular edge that rounds the lower edge and the pointed upper tip (Wagner et al. 2020).



Figure 3. *Gouania adriatica* Wagner, Kovačić and Koblmüller, 2020, taken from Wagner et al. (2020). Photos by M. Wagner and M. Kovačić.

1.2. Microhabitat niche partitioning

Microhabitat niche partitioning is common in several small-bodied cryptobenthic taxa, whereas coexisting competing species are able to exploit a wider range of food, substrates or habitats compared to larger fishes (e.g. Yamada et al. 2008; Tornabene et al. 2013; Ahamdia et al. 2018; Brandl et al. 2018, 2020). Thus far, most microhabitat association studies in cryptobenthic fish were conducted in tropical seas (e.g. the Great Barrier Reef in Australia), whereas the investigated species exhibited spatial fine-scale partitioning across the shelf

(Tornabene et al. 2013; Goatley et al. 2016) or even within different host coral species (Dirnwöber and Herler 2007). In the Mediterranean, some cryptobenthic species from families Tripterygiidae and Gobiidae can be linked to different depth ranges suggesting a certain level of space partitioning (La Mesa et al. 2005). Nonetheless, niche partitioning is considered a key factor in sympatric speciation of *G. pigra* and *G. adriatica* (Wagner et al. 2019, 2020).

Gouania's small size (< 6cm), elongated body with a high number of vertebrae, small eyes and the adhesive disc are particular morphological adaptations which enabled them to invade the interstices of intertidal pebble beaches (Hofrichter and Patzner 2000; Wagner et al. 2019). The interstitial of pebble beaches can be considered very demanding environments for vertebrates, whereas waves, tides and heavy tourist pressure are predominant factors (Wagner et al. 2019). Unsurprisingly, as mentioned before, only *Gouania* and Pacific gobies from the genus *Luciogobius* have successfully adapted to life-hostile intertidal pebble beaches (Yamada et al. 2009; Wagner et al. 2019). Remarkably, both genera independently converged to the same morphological adaptations (i.e. excessive vertebral segmentation, elongated, scale-less body, and reduced fins), which indicate strong selective pressures in these environments. For instance, the elongated body shape, induced by increased number of vertebrae, elevates the body flexibility and allows the locomotion in the interstices of the gravel sediment (Yamada et al. 2009; Wagner et al. 2019). Additionally, these specific adaptations have allowed microhabitat specialization and fine-scale niche utilization in *Luciogobius* (Yamada et al. 2009). If similar trajectories hold true for sympatric occurring *Gouania* species remains to be explored, but previous studies suggested that the slender-bodied (number of vertebrae 35-38, smaller eyes), *G. pigra* is usually found in finer gravel of pebble beaches compared to its sympatric stout-bodied (number of vertebrae 35, larger eyes) congener, *G. adriatica* (Wagner et al. 2019, 2020).

1.3. Aim of this thesis

The aim of this thesis is to determine whether slender-bodied *G. pigra* and stout-bodied *G. adriatica*, occurring in the same area, inhabit different microhabitats. Based on the two extremely divergent morphospaces that the species occupy, I hypothesise that gravel size

could be a good proxy for determination of the microhabitats and therefore, niche partitioning between the two species, is very likely (see also Wagner et al. 2019, 2020).

Additionally, in Croatia, the biocenosis of infralittoral gravels is listed in all three *Annexes of Regulation of habitat types, habitat maps, threatened and rare habitat types and measures for the conservation of habitat types* (Narodne novine 88/2014). This should prevent further degradation and ensure the retention of a favourable state of conservation in terms of national and European importance. Therefore, the recent description of the two new Adriatic species, one of which is endemic to the Adriatic basin, fosters a critical look on the conservation of these habitats. Therefore, this thesis also aims to focus on the geological characteristics and habitat structure of infralittoral gravel beaches. This work will therefore deliver a baseline for future investigations in this unassuming habitat that, of all known vertebrate species in Europe, is exclusively occupied by species of the genus *Gouania*.

2. RESEARCH AREA

The Croatian coastline is characterized by a high, steep and rocky carbonate coast, formed after the last sea-level rise by the submerging of the previously karstified carbonate relief (Pikelj and Juračić 2013). Beaches are rare along the coast and mostly developed as gravel pocket beaches in carbonates or mixed gravelly-sandy beaches formed in flysch rock assemblages (Pikelj and Juračić 2013). In this study, I investigated 11 beaches in total, which are all located on the Istrian peninsula. Whereas the majority of these beaches are located within the urban area of Pula on the western Istrian coast, two (Cava and Sveta Marina) are remote beaches situated on the eastern Istrian coast (**Figure 4**).

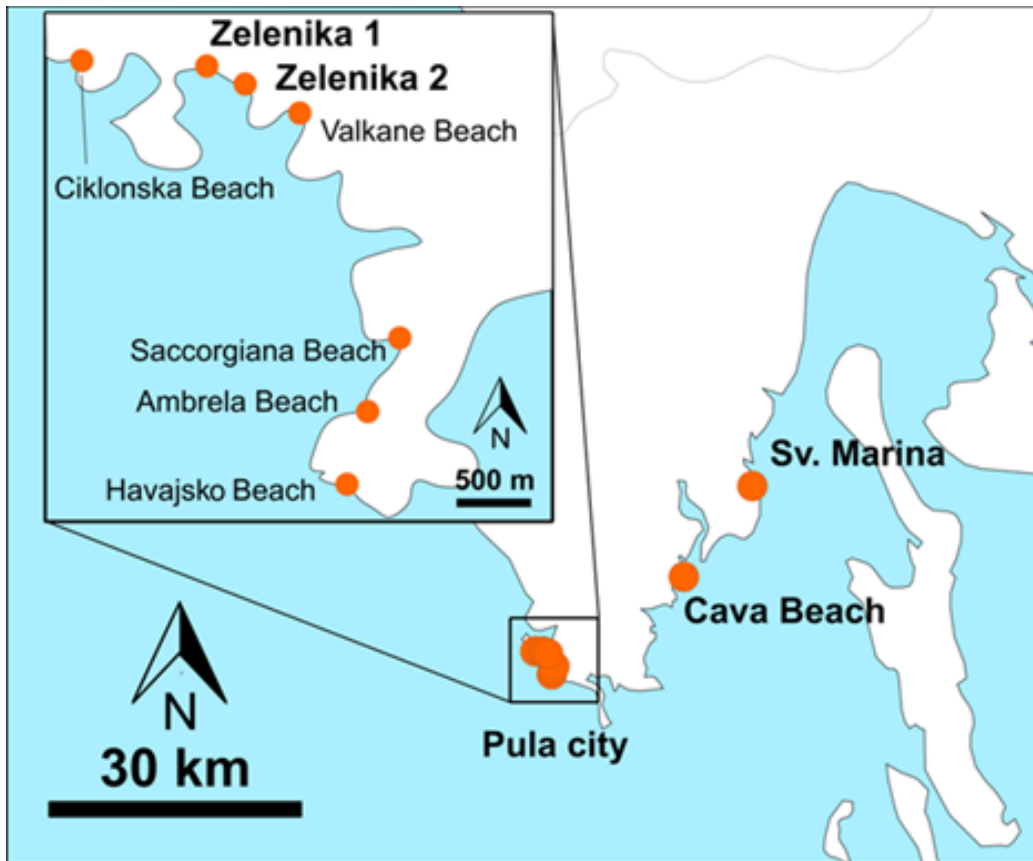


Figure 4. Map showing locations in which *Gouania* were present. The four beaches included in the granulometric analysis Cava, Sveta Marina, Zelenika 1 and Zelenika 2 are shown in bold.

Despite investigating the occurrence of the newly described and redescribed *Gouania* species in all 7 above-mentioned beaches in Pula, only Zelenika 1 (**Figure 5**) and Zelenika 2 (**Figure 6**) were included for the comparative sedimentological analyses. These beaches consist of carbonate material which lies above the low rocky carbonate coast. The original lithology of this coast is characterized by the Lower Cretaceous thin-bedded limestones intercalated by the rare lenses of dolomites, marls, and breccias ($_{1K1}^5$) (Polšak 1967). The structure of the underlying rocks is visible in the submarine part of the beach.



Figure 5. Sampling site Zelenika 1. Photo by M. Pamić.



Figure 6. Sampling site Zelenika 2. Photo by M. Pamić.

In contrast, the eastern Istrian coast is steep, rocky, and much higher compared to the western coast. Both beaches, Cava (**Figure 7**) and Sveta Marina (**Figure 8**), are typical natural pebble pocket beaches, formed at the end of the recent and/or subrecent surface water streams (Pikelj and Juračić 2013) and both oriented eastwards. The subaerial and submarine parts of the beaches are covered by natural beach sediment. Well-bedded grey Upper Cretaceous limestones (${}_{1}K_2^3$) underlie Cava beach, (Šikić et al. 1969), while Lower Cretaceous grey and brown thick bedded limestones (K_2^2-3) (Magaš 1968) underlie Sv. Marina beach.



Figure 7. Sampling site Cava. Picture by F. Keller
(<https://goo.gl/maps/g6cUKmdFZBsMmNsQ6>).



Figure 8. Sampling site Sveta Marina. Inspection of the beach for *Gouania*'s presence. Photo by M. Wagner.

3. MATERIALS AND METHODS

3.1. Sampling

In a preliminary study, from March to June 2020, I examined 11 gravel beaches in Pula. However, for the comparative analyses, I included only the sites at which both species were present and highly abundant. Therefore, from September to October 2020, I investigated two sites in Pula (i.e. Zelenika 1 and Zelenika 2) and two more sites on the eastern Istrian coast (Sveta Marina and Cava bay). I sampled in conditions without strong wind and regardless of the height of the tides and the presence of swimmers. I took the samples by pulling a customized iron bucket through the gravel to a depth of 50 cm (**Figure 9**).



Figure 9. Customized iron bucket facilitates sampling through the gravel. Design by M. Wagner and M. Pamić. Photo by M. Pamić.

After each trial, I separated the collected *Gouania* and measured their total length in millimetres (using a digital calliper Iskra) and I determined the sex (if possible) as well. If more than one specimen or different species were in the same bucket, I assigned the same bucket to each specimen. Species identification was done based on the key provided by Wagner et al. (2020). Following this, I released the specimens far enough from the original sampling site to ensure that the same individuals are not re-captured. I performed statistical analysis and comparisons between species using descriptive statistics, chi-squared test and Mann–Whitney U test (after testing for normality of the gathered data using the Shapiro–Wilk test). I did all analyses in R Ver. 4.0.3 language and environment for statistical computing and graphics (R Core Team 2020).

3.2. Granulometrics

Sieve analysis (granulometrics) is one of the oldest and most common techniques used to obtain the particle size distribution (Dishman 2006). Above all, its application is simple, reproducible and inexpensive. After collecting gravel with the above-mentioned bucket (**Figure 9**), the pebbles were separated through a customized sieve apparatus using 5 fractions that roughly represent the range of pebbles that are habitable for *Gouania*. The exact mesh apertures of each fraction were 46.15 mm, 25 mm, 12.80 mm, 5.51 mm and 1.51 mm, respectively. However, later in the text, I will refer to them as rounded centimetre values equal to 5, 2.5, 1.3, 0.5 and 0.1. In order to ensure a proper separation of different grain sizes, I washed and shook the sieve apparatus properly. After separation, I weighted each sieve, full (including content) and empty (without content), whereas the total weight of each fraction was calculated as the difference of these two measures. Following this, I calculated the relative masses of individual fractions for each sample, which was used for the statistical analysis. To infer differences of sediment compositions between species *G. pigra* and *G. adriatica*, I performed a multivariate principal component analysis (PCA) and a Mann–Whitney U test in R Ver 4.0.3 (R Core Team 2020). I used Shapiro–Wilk test to test normality of the data.

Additionally, I calculated the retained mass data using GRADISTAD Ver. 8 (Blott and Pye 2001). Through this program I obtained the mean, median, sorting index, skewness and kurtosis. Grain sizes in Φ units gotten from the cumulative curve at cumulative percentages: 5, 16, 25, 50, 75, 84 and 95 were used for the calculation of granulometric parameters. For calculating the correlations with the body size of the species and sediment composition, the mean, median and sorting index and differences between locations were used and calculated in R Ver. 4.0.3 (R Core Team 2020). The difference between locations in granulometric properties was tested using Kruskal-Wallis test. For results that were statistically significant, Dunn’s test was performed with a Benjamini-Hochberg p-adjustment method to determine exactly which locations were different. Statistical formulas used in GRADISTAD Ver. 8 (Blott and Pye 2001) for the calculation of grain size parameters and their descriptions were (Folk & Ward, 1957):

Median (medium grain size) represents the value of which 50% of the particles are larger and 50% smaller:

$$Md = \Phi 50$$

Mean (average grain size):

$$Mz = \frac{\Phi 16 + \Phi 50 + \Phi 84}{3}$$

Sorting shows the uniformity of distribution:

$$So = \frac{\Phi 84 - \Phi 16}{4} + \frac{\Phi 95 - \Phi 5}{6,6}$$

Sediment sorting categories:

< 0,35.....	Very well sorted
0,35-0,50.....	Well sorted
0,50-0,70.....	Moderately well sorted
0,70-1,00.....	Moderately sorted
1,00-2,00.....	Poorly sorted
2,00-4,00.....	Very poorly sorted
> 4,00.....	Extremely poorly sorted

Skewness (the asymmetry of the distribution) shows deviations in the distribution of particles. Positive values indicate that the larger fraction predominates, i.e. that the curve is inclined towards the smaller sediments, and negative values show that the smaller fraction prevails, i.e. that the curve is inclined towards the larger sediments:

$$Sk = \frac{\Phi 16 + \Phi 84 - 2\Phi 50}{2(\Phi 84 - \Phi 16)} + \frac{\Phi 5 + \Phi 90 - 2\Phi 50}{2(\Phi 95 - \Phi 5)}$$

Skewness categories:

-1,00 to -0,30	Very coarse skewed
-0,30 to -0,10	Coarse skewed
-0,10 to 0,10	Symmetrical
0,10 to 0,29.....	Fine skewed
0,30 to 1,00.....	Very fine skewed

Kurtosis refers to the scattering of the distribution. If the curve is sharpened (thinner tailed), the material is distributed around one grain size, and if it is flattened (fatter tailed), several granulometric fractions are present.

$$Kg = \frac{\Phi 95 - \Phi 5}{2,44(\Phi 75 - \Phi 25)}$$

Kurtosis categories:

< 0,67.....	Very platykurtic
0,67-0 90.....	Platykurtic
0,90-1,11.....	Mesokurtic
1,11-1,50.....	Leptokurtic
1,50-3,00.....	Very leptokurtic
> 3,00.....	Extremely leptokurtic

4. RESULTS

4.1. Sampling and body length of species

Of the eleven examined gravel beaches in Pula, *G. pigra* was present without its sympatric congener *G. adriatica*, at three different beaches (Havajsko Beach, Ambrela Beach and cove Saccorgiana). However, both species were found in sympatry at Ciklonska Beach, Valkane Beach and the beaches Zelenika 1 and Zelenika 2. Of these four locations, only Zelenika 1 and Zelenika 2 as well as the eastern Istrian locations Cava bay and Sv. Marina were investigated and included into the comparative granulometric and niche partitioning analyses. During sampling in Sveta Marina, no specimens of *G. adriatica* were found, but *G. pigra* occurred in high frequencies. Previous studies also confirm this observation, since *G. adriatica* was rarely found at this site (Wagner M.; personal communication). Most sampled specimens were collected at Zelenika 2 (N=47), followed by Zelenika 1 (N=30). A summary of all numbers of individuals of each species by location is show in **Table 2**. Difference in frequency between species by location was tested using chi-squared test. Frequency of *G. adriatica* significantly differs from *G. pigra*'s on these four locations ($\chi^2 = 10.13$, $P = 0.0175$).

Table 2. Number of *G. adriatica* and *G. pigra* individuals by location.

Species	Location			
	Cava	Sv. Marina	Zelenika 1	Zelenika 2
<i>G. adriatica</i>	2	0	16	22
<i>G. pigra</i>	14	8	14	25

Morphometric data were collected from a total of 101 individuals from the two species, *G. pigra* and *G. adriatica*. Since sampling was carried out during autumn, sex determination was difficult with just visual inspection, therefore, there are no data of 32 individuals. Descriptive statistics for total length are shown in **Table 3**. According to this, the minimum and the maximum length of both *G. adriatica* and *G. pigra* were recorded at Zelenika 2. The population of *G. adriatica* at Cava was the largest in size, while at Sv. Marina, *G. pigra* had the smallest maximum and average body size.

Table 3. Descriptive statistics with highlighted minimum (min), maximum (max), mean values (\bar{x}), standard deviation (StdDev) of measure and locations. Sizes are expressed in millimetres (mm).

Species	Total length	Location			
		Cava	Sv. Marina	Zelenika 1	Zelenika 2
<i>G. adriatica</i>	min	29,93		23,15	21,04
	max	48,42		50,84	52,36
	\bar{x}	39,16		34,72	32,71
	StdDev	13,07		8,59	10,12
<i>G. pigra</i>	min	29,51	28,27	27,81	16,24
	max	48,33	45,92	51,78	54,34
	\bar{x}	39,51	34,86	42,41	41,92
	StdDev	5,14	6,89	6,91	8,74

The difference in size between *G. adriatica* and *G. pigra* was significant ($P = 0.00046$) by means of a Mann–Whitney U test. Furthermore, Zelenika 1 and Zelenika 2 showed significant difference in size between the two species with ($P \leq 0.05$; **Figure 10**), whereas Sveta Marina and Cava bay were excluded from these analyses (due to a lack of *G. adriatica*).



Figure 10. Summary statistics and P values of Mann–Whitney U test for size of *G. adriatica* and *G. pigra* by location (Z1= Zelenika 1, Z2= Zelenika 2).

4.2. Relative mass comparison

A total of 52 buckets with 101 specimens were investigated. One bucket corresponded to one sample, therefore, 52 samples were inspected, weighted and used for granulometrics. Descriptive and differential statistics (using Mann–Whitney U test) for the total relative mass are displayed in **Figure 11**. The statistical test revealed no significant difference ($P \geq 0.05$) when comparing the relative sediment mass between species for each sieves, independent of the sampling site. Sieve fractions that comprise most of the sediment were 5, 2.5 and 1.3 cm.

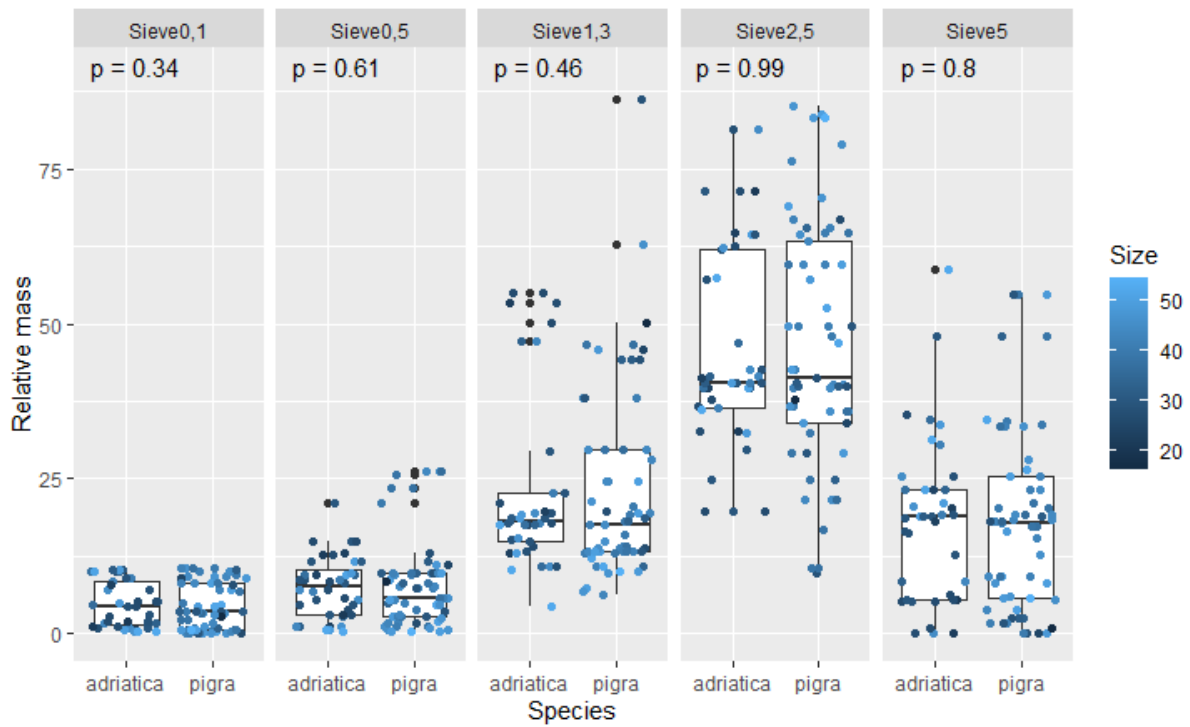


Figure 11. Summary statistics and P values of Mann–Whitney U test for total relative mass between species for individual sieves.

When comparing the different relative masses for each sieve fraction between species in different locations, only Zelenika 2 revealed significant results (**Figure 12**) at sieve fractions 1.3 and 2.5 (Mann–Whitney U test; $P_{1.3}=0.031$, $P_{2.5}=0.043$).

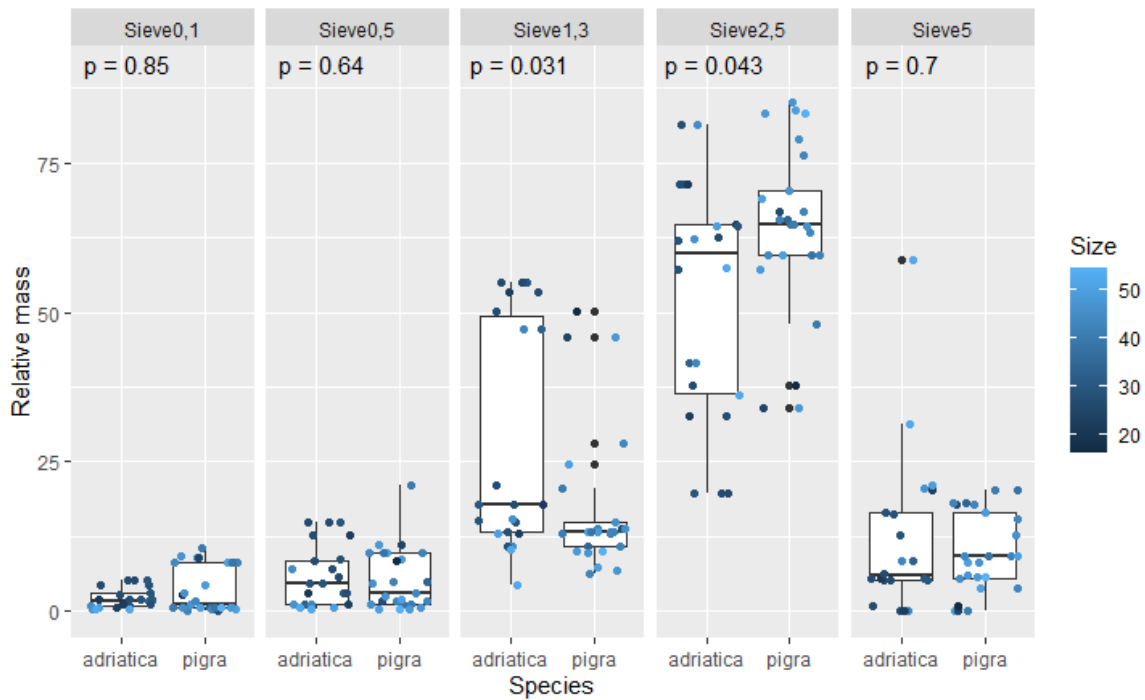


Figure 12. Summary statistics and *P* values of Mann–Whitney U test of relative mass between species by individual sieves for location Zelenika 2.

The multivariate principal component analysis (PCA) (**Figure 13**) graphically confirmed the previously described results; i.e. the axis with most explained variation could not separate the two species. The first principal component (PC) accounts for 42.35% of the total variance in the dataset, the second PC for 28.79% and the third PC 19.47%. Therefore, the first three principal components account for approximately 90% of the explained variance in the dataset. The first component represents the relative mass of the sieve fraction 2.5 and 0.5, which are inversely correlated. The second component represents the relative mass of the sieve fraction 5, which is negatively correlated with the sieve fraction 1.3.

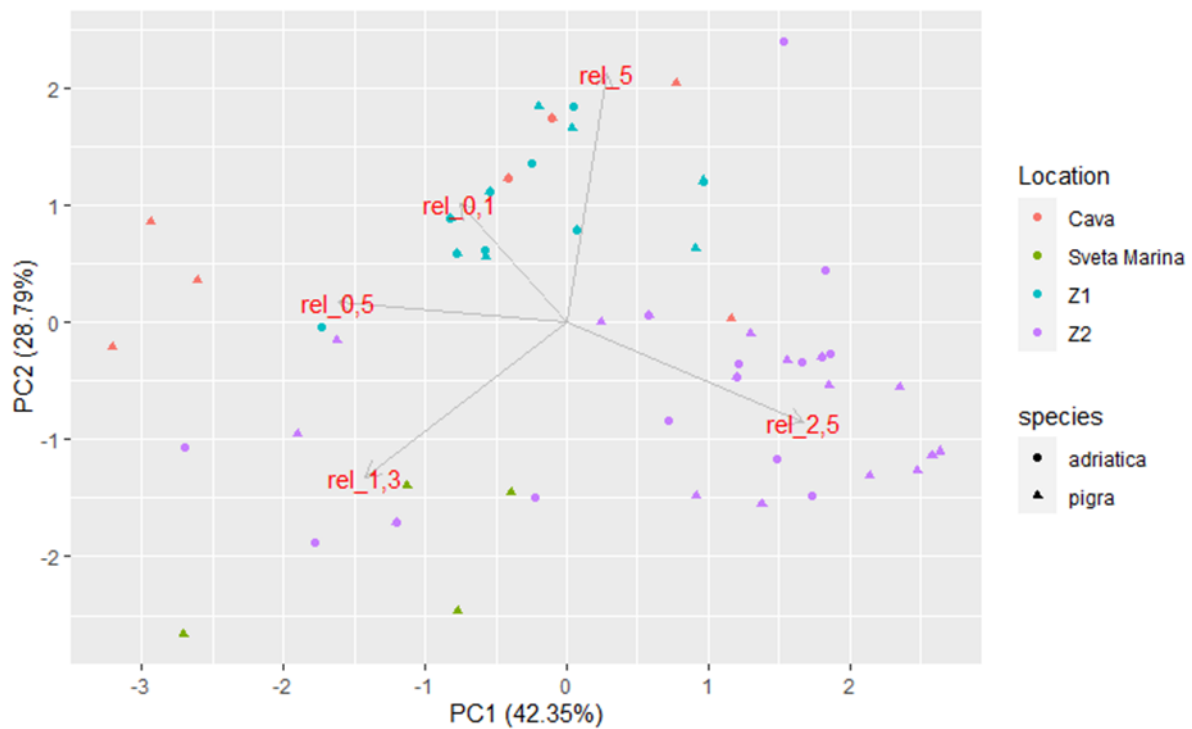


Figure 13. Graphical representation of PCA analysis for total relative mass. The value of PC 1 is represented on the x axis and the value of PC 2 on the y axis. In red are the names of the vector loadings - relative mass of the sieve fraction 5 (rel_5), relative mass of the sieve fraction 2.5 (rel_2.5), relative mass of the sieve fraction 1.3 (rel_1.3), relative mass of the sieve fraction 0.5 (rel_0.5), relative mass of the sieve fraction 0.1 (rel_0.1).

Since the differences in the sediment composition between the two species were exhibited in Zelenika 2 and the difference in size was statistically significant in Zelenika 1 and Zelenika 2 (**Figure 10**), further analyses were conducted based on individuals larger than 40 mm (**Figure 14**) and 45 mm (**Figure 15**). However, for adults larger than 40 mm no significant difference in relative mass between the two species was detected (Mann–Whitney U test, $P \geq 0.05$), the confined dataset to individuals larger than 45 mm showed significant results at a sieve size of 5 cm (Mann–Whitney U test; $P = 0.04$).

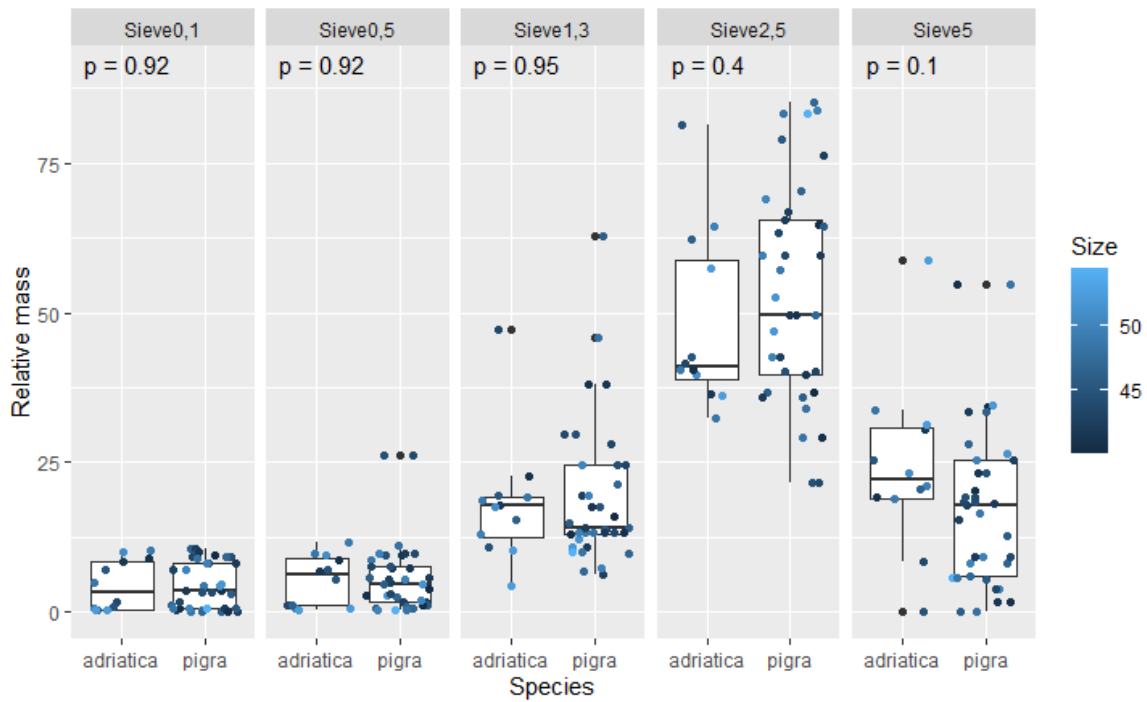


Figure 14. Summary statistics and P values of Mann–Whitney U test for relative mass of individual sieves between *G. adriatica* and *G. pigra* larger than 40 mm.

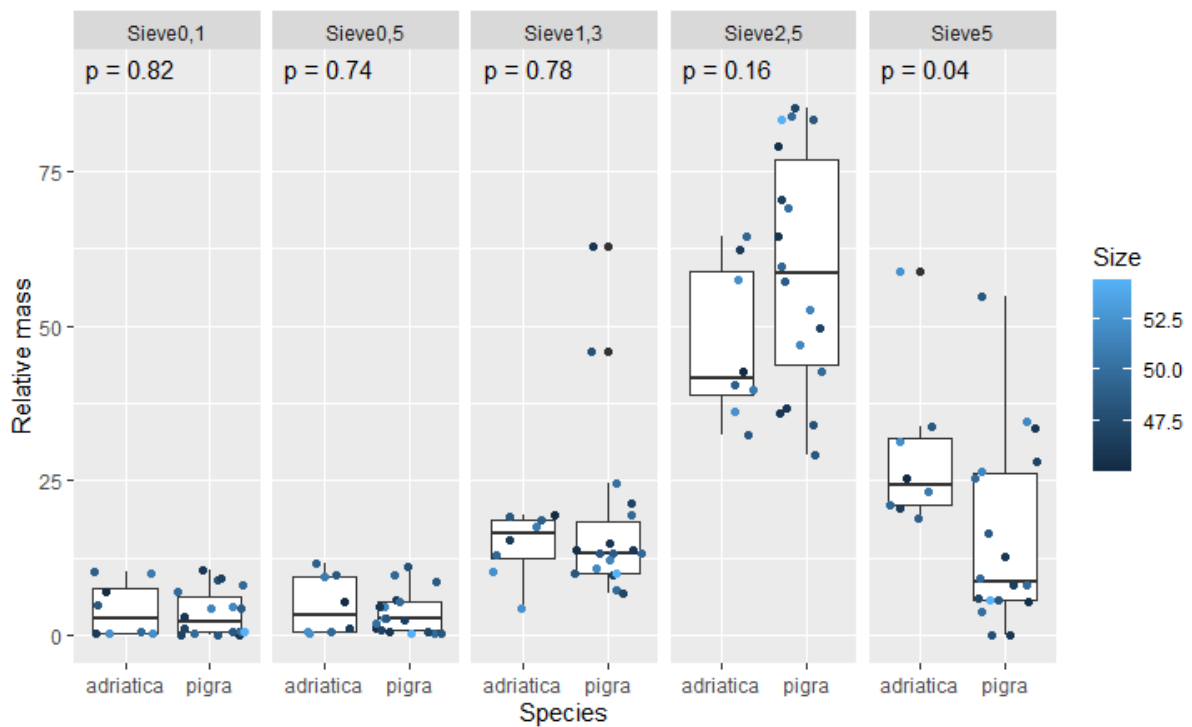


Figure 15. Summary statistics and P values of Mann–Whitney U test for relative mass of individual sieves between *G. adriatica* and *G. pigra* larger than 45 mm.

4.3. Sieve analysis

Sieve analysis results obtained from GRADISTAT for each sample are shown in **Table 4**. All 52 samples belonged to the gravel textural group with more than 97% sediment consisting of gravel and 3% of sand. There was no mud present in the samples. 63% of all samples were very well sorted and 15% were moderately well sorted. This shows that most of the sampled sediment has undergone excessive sediment transport by sea water. More than 60% of samples were very coarse and coarse skewed, meaning that larger sediments prevailed. Furthermore, 60% of all samples were very platykurtic, 8% were platykurtic and 19% were mesokurtic. This implies that almost 70% of all samples were composed of several granulometric fractions.

Table 4. Granulometric parameters and corresponding descriptions for 52 samples.

Sample	Mz (μm):	Md (μm):	So Φ	Sk Φ	Kg Φ	SORTING:	SKEWNESS:	KURTOSIS:
1	14522,7	35410,6	-0,432	-6,596	0,927	Very Well Sorted	Very Coarse Skewed	Mesokurtic
2	13699,1	34853,7	-0,329	-8,375	1,880	Very Well Sorted	Very Coarse Skewed	Very Leptokurtic
3	22338,9	22286,3	0,803	0,145	1,059	Moderately Sorted	Fine Skewed	Mesokurtic
4	23943,4	25342,9	0,781	0,292	1,051	Moderately Sorted	Fine Skewed	Mesokurtic
5	18295,1	18295,1	0,400	-0,136	1,015	Well Sorted	Coarse Skewed	Mesokurtic
6	22598,2	21671,9	0,558	-0,157	0,782	Moderately Well Sorted	Coarse Skewed	Platykurtic
7	27207,7	32195,9	0,249	-3,178	-0,408	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
8	29180,2	34258,6	-0,241	0,051	-1,235	Very Well Sorted	Symmetrical	Very Platykurtic
9	32668,1	32668,1	0,356	0,534	0,799	Well Sorted	Very Fine Skewed	Platykurtic
10	34973,1	34973,1	-0,160	-0,918	-1,666	Very Well Sorted	Very Coarse Skewed	Very Platykurtic

Continuation of **Table 4**

11	18208,1	23411,2	1,214	0,497	0,916	Poorly Sorted	Very Fine Skewed	Mesokurtic
12	34006,1	34006,1	0,044	-1,431	-0,692	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
13	18636,4	24004,6	0,184	-1,737	-0,309	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
14	20897,5	28515,2	0,295	-32,372	-0,03	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
15	14832	33274,8	-0,331	-6,755	-1,089	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
16	17317,8	32424,6	-0,223	-7,722	-0,309	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
17	14255,3	36049,2	-0,438	-7,02	0,59	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
18	15856,2	30552,1	0,091	-8,138	-0,091	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
19	16849	37765,3	-0,743	-2,888	0,952	Very Well Sorted	Very Coarse Skewed	Mesokurtic
20	26199,9	34832,8	-0,462	-3,011	-1,527	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
21	25127,5	24956,7	0,293	-2,775	-0,159	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
22	18392,7	18434,6	0,857	0,121	1,343	Moderately Sorted	Fine Skewed	Leptokurtic
23	14109,2	34015,4	-0,537	-3,735	5,592	Very Well Sorted	Very Coarse Skewed	Extremely Leptokurtic
24	18604,1	35175,8	-0,735	-2,095	-4,812	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
25	17928,1	18158,3	0,336	-2,053	-0,334	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
26	15932,2	20342,6	0,214	-1,781	-0,249	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
27	13614,1	33157,5	-1,257	-1,507	0,57	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
28	13772,2	43930,3	-0,93	-2,594	0,618	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
29	13697,9	19071,7	0,215	-3,306	-0,138	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
30	28591,2	30622,5	0,557	0,296	1,021	Moderately Well Sorted	Fine Skewed	Mesokurtic

Continuation of **Table 4**

31	18595,5	19793,1	1,039	0,281	1,403	Poorly Sorted	Fine Skewed	Leptokurtic
32	30204,4	31670	0,583	0,395	1,727	Moderately Well Sorted	Very Fine Skewed	Very Leptokurtic
33	29429,9	31497,4	0,508	0,554	0,983	Moderately Well Sorted	Very Fine Skewed	Mesokurtic
34	25301,6	35095,1	-0,523	-2,401	-1,589	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
35	28807,7	32072,9	0,597	1,59	1,155	Moderately Well Sorted	Very Fine Skewed	Leptokurtic
36	22015,2	21549,7	0,752	0,087	1,062	Moderately Sorted	Symmetrical	Mesokurtic
37	26957,6	30088,8	0,628	0,964	0,809	Moderately Well Sorted	Very Fine Skewed	Platykurtic
38	33635,8	33635,8	0,249	1,492	0,38	Very Well Sorted	Very Fine Skewed	Very Platykurtic
39	27264,1	29506,1	0,599	0,288	0,871	Moderately Well Sorted	Fine Skewed	Platykurtic
40	18250,9	28807,2	0,4	-84,215	-0,01	Well Sorted	Very Coarse Skewed	Very Platykurtic
41	19841,8	28931,5	0,272	-17,346	-0,053	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
42	18884,9	35063,9	-0,468	-3,535	-1,284	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
43	19862,8	28762,5	0,377	-12,632	-0,067	Well Sorted	Very Coarse Skewed	Very Platykurtic
44	24205,1	30318,6	0,691	2,219	0,48	Moderately Well Sorted	Very Fine Skewed	Very Platykurtic
45	29945,7	32399,6	0,199	-3	-0,469	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
46	20832,2	20124	0,734	0,032	1,081	Moderately Sorted	Symmetrical	Mesokurtic
47	33315,4	33315,4	0,293	0,855	0,57	Very Well Sorted	Very Fine Skewed	Very Platykurtic
48	30097,4	33076,1	0,047	-1,474	-0,895	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
49	25353,7	34523,6	-0,448	-3,595	-1,465	Very Well Sorted	Very Coarse Skewed	Very Platykurtic
50	13018,9	25863,6	-1,485	-0,948	0,613	Very Well Sorted	Very Coarse Skewed	Very Platykurtic

Continuation of **Table 4**

51	19184,9	37821	-0,956	-1,644	2,953	Very Well Sorted	Very Coarse Skewed	Very Leptokurtic
52	30106,7	34348,9	-0,276	-0,131	-1,466	Very Well Sorted	Coarse Skewed	Very Platykurtic

In general, the difference in mean between locations was statistically significant (Kruskal-Wallis test; $P = 5.8e-14$). The mean of Cava was different from the mean of Sveta Marina and Zelenika 2 (Dunn's test: $P_{SM}=0.0006$, $P_{Z2}=0.0000$). Furthermore, Zelenika 1 and Zelenika 2, as well as Zelenika 1 and Sveta Marina, were statistically different (Dunn's test: $P_{Z2}=0.0000$, $P_{SM}=0.0098$).

Across different sampling sites the median differed significantly (Kruskal-Wallis test; $P = 0.013$) (**Figure 16**). Post-Hoc Dunn's test showed that the median of Sveta Marina statistically differed from the median of all other locations ($P_{Cava}=0.0054$, $P_{Z1}=0.0039$, $P_{Z2}=0.0064$).

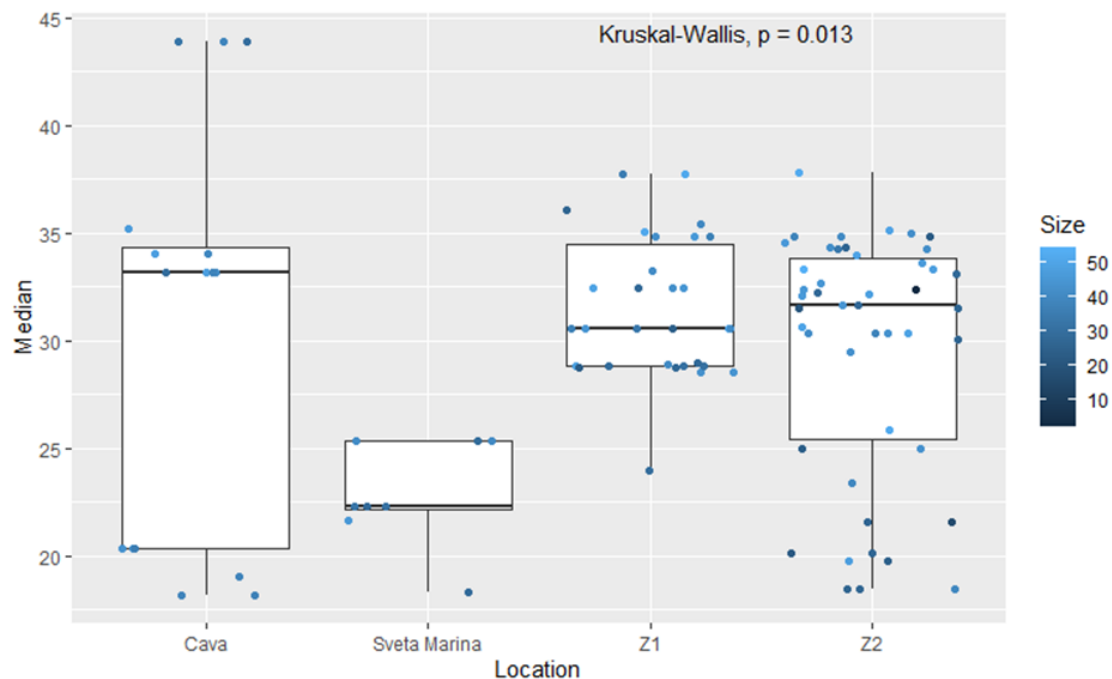


Figure 16. Summary statistic and P value of Kruskal-Wallis test for the median between locations (Z1= Zelenika 1, Z2= Zelenika 2). The size of the median and the sizes of individuals are expressed in millimetres (mm).

Additionally, the sorting index showed statistical differences between all locations (Kruskal-Wallis test; $P = 2.6e-7$). The sorting index of Zelenika 2 compared to the remaining three locations was significant ($P_{Cava}=0.0000$, $P_{SM}=0.0081$, $P_{Z1}=0.0040$) and the sorting index of Sveta Marina differed from Cava ($P = 0.0000$) and Zelenika 2 ($P = 0.0001$), but not between Zelenika 1 and Cava. The skewness and kurtosis between locations were significant (Kruskal-Wallis test; $P_{sk}=2.9e-14$, $P_{kg}=0.044$). As for the skewness, the post-hoc (Dunn's) test revealed that locations Sveta Marina, Zelenika 1 and Zelenika 2 differ from Cava and that Zelenika 1 statistically differs from Sveta Marina and Zelenika 2 (all $P \leq 0.05$). Furthermore, the kurtosis of Sveta Marina differs from the kurtosis of all other locations (Dunn's test; $P_{Cava}=0.0206$, $P_{Z1}=0.0145$, $P_{Z2}=0.0235$).

The correlation between body size and all granulometric parameters was calculated. The correlation between the size and the mean (**Figure 17**) for *G. adriatica* was partly and negatively correlated (Pearson's correlation coefficient; $R = -0.35$ and $P = 0.026$) and no correlation was detected for *G. pigra* ($R = 0.21$, $P = 0.1$). On the contrary, the positive correlation between the size and the median (**Figure 18**) was not significant for *G. adriatica* ($R = 0.17$, $P = 0.29$), but it was for *G. pigra* ($R = 0.33$, $P = 0.01$). Interestingly, independent of the species, most of the individuals were localised between the median of 30 and 35.

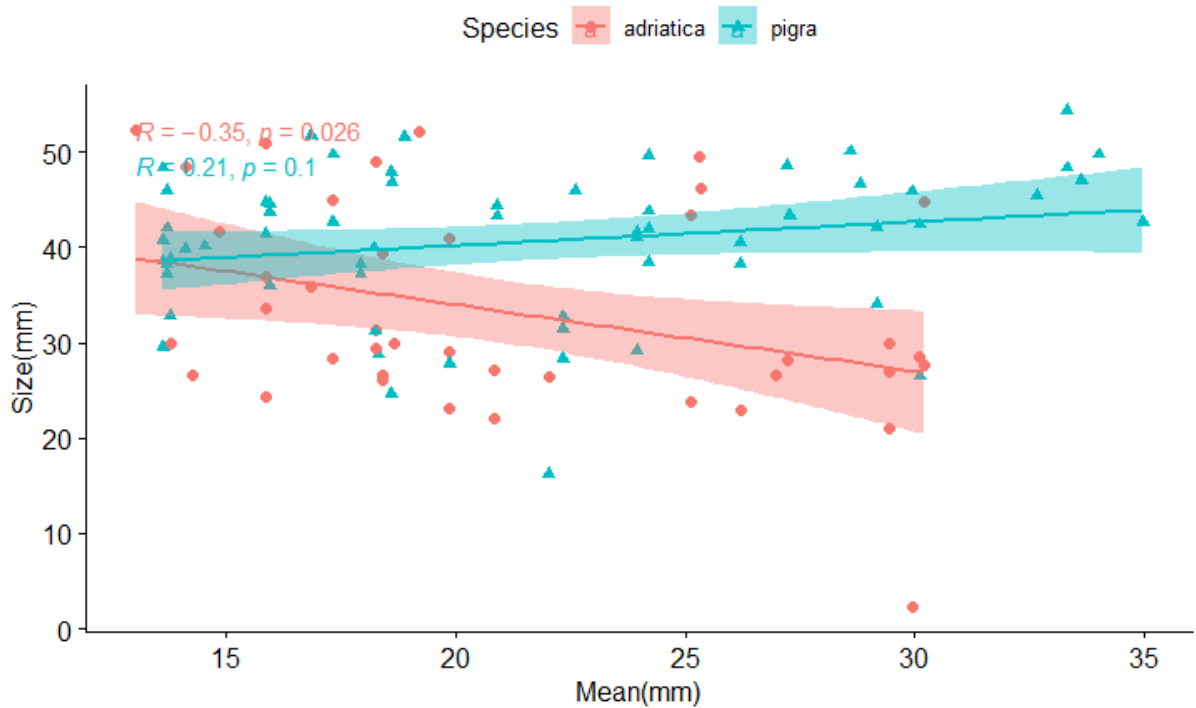


Figure 17. Scatter plot showing correlation of fish size and granulometric mean for both species with their corresponding Pearson's correlation coefficients and *P* values.

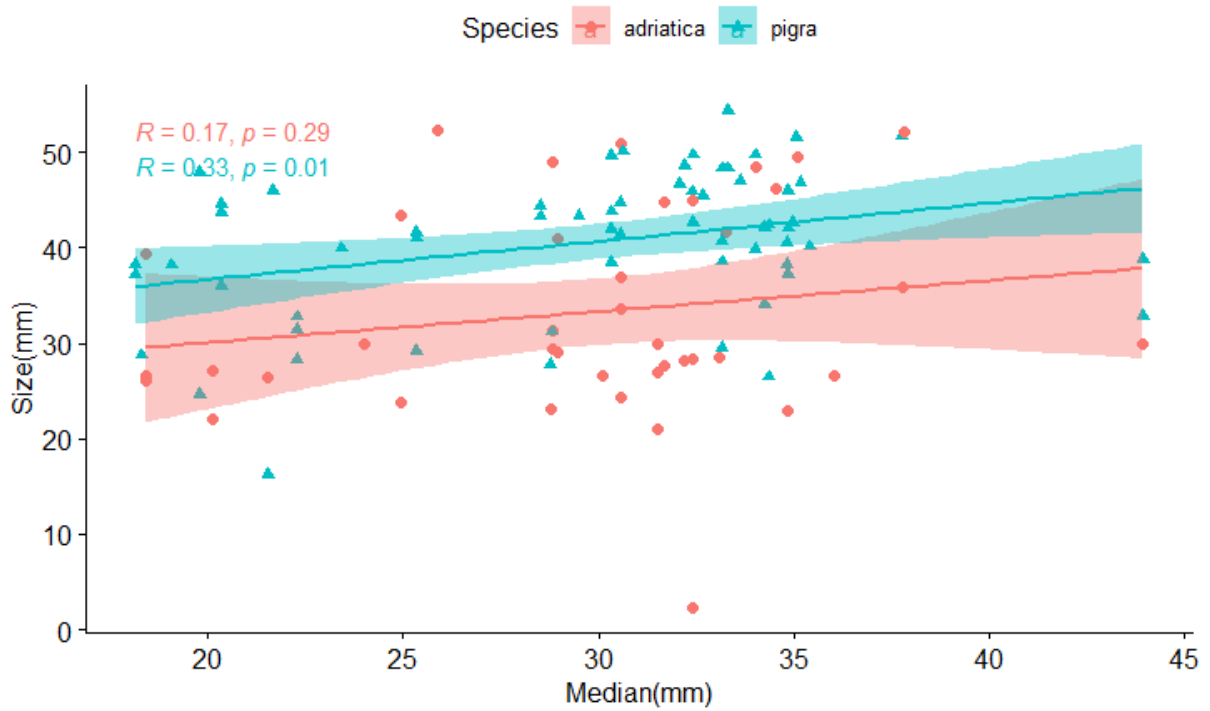


Figure 18. Scatter plot showing correlation of fish size and granulometric median for both species with their corresponding Pearson's correlation coefficients and *P* values.

The correlation between the size of the species and the sorting index (**Figure 19**) revealed a similar picture like the mean. Hence, the size of *G. adriatica* was significantly negatively correlated with the sorting index ($R = -0.45$, $P = 0.0037$), but not for *G. pigra* ($R = -0.13$, $P = 0.31$). Notably, sorting index values greater than 1 represent a poorly sorted sediment and values smaller than 0.5 a well sorted sediment. Lastly, the skewness and kurtosis were not statistically correlated with either *G. adriatica* or *G. pigra* ($P_{ska} = 0.51$, $P_{skp} = 0.44$; $P_{kga} = 0.38$, $P_{kgp} = 0.34$).

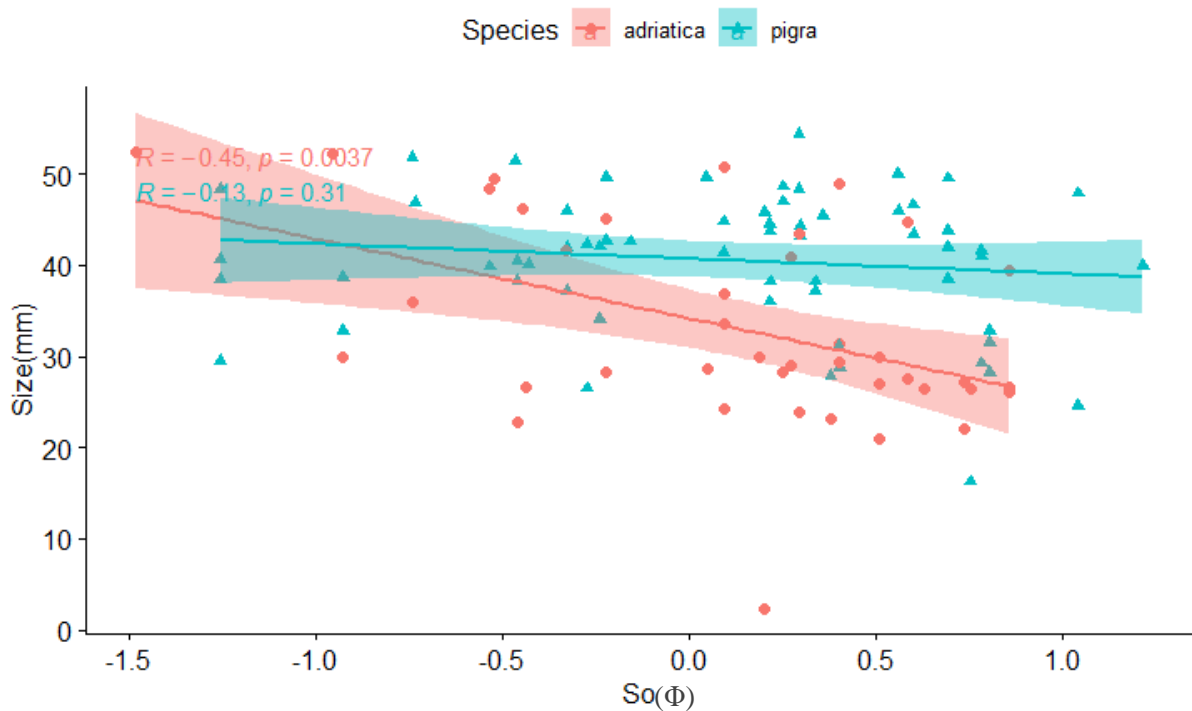


Figure 19. Scatter plot showing correlation of size and sorting index (So) for both species with their corresponding Pearson's correlation coefficients and P values.

5. DISCUSSION

5.1. Microhabitat niche partitioning in *Gouania*

For many cryptobenthic fishes, the microhabitat niche partitioning was a major driver of their diversification and this can be likely linked to their small size and their association to the

benthos (Brandl et al. 2018). The small size enables these fishes to feed on a variety of prey items and inhabit small spatial niches, inaccessible for larger fishes (Brandl et al. 2018). The results of the analysis of total length for species *G. pigra* and *G. adriatica* matched the records from literature (Wagner et al. 2020). Nevertheless, unexpectedly large specimens were recorded in Zelenika 1 and 2, with lengths in both species exceeding 50 mm. Most of these individuals were males. In clingfishes, males tend to have larger and more bulky body shapes (particularly in the head region) compared to females (Briggs 1955) and this holds true for *Gouania* as well (Hofrichter 1995). Due to the simultaneous mating seasons, an overlap between the temporal niches most likely causes migration of the males towards larger pebbles in search for suitable mating spots (own observation; Gonç alves et al. 2002). Also, males presumably guard the nests (own observation), which is a form of parental care that has been previously observed in other Gobiesocidae species (Coleman 1999; Gonç alves et al. 2002; Pires and Gibran 2011). The nests of *G. pigra* are located in the upper layer of the pebbles, whereas the *G. adriatica*'s nests are located in the deeper areas (own observation). Future studies on the territoriality and breeding ecology are needed for better understanding of these newly described species.

Overall, the examination of species occurrences showed that on the locations Zelenika 2 and Zelenika 1, almost the same number of individuals of Adriatic blunt-snouted clingfish and piglet sucker were caught. From the total of 101 specimens, 47 originated from Zelenika 2, which amounts to 47% of the whole sample, and 30% of all samples were from Zelenika 1. Hence, it seems that these locations could provide clearer, more accurate understanding of species habitat preferences compared to Sv. Marina and Cava bay. In the case of microhabitat partitioning of *Luciogobius*, which inhabits a similar environment as *Gouania*, the habitat preferences of different species were associated with the number of vertebra and the body size (Yamada et al. 2009). Therefore, similar to the phenotype-environment correlation in *Luciogobius*, we would expect that stout-bodied *G. adriatica*, which has a lower number of vertebrae (35), should prefer coarser gravel, whereas slender-bodied *G. pigra* with larger number of vertebrae (35-38) should prefer finer gravel. Even though, the comparison of the total relative mass of five sieve fractions between *G. adriatica* and *G. pigra* did not reveal a clear partitioning of microhabitats. Further investigations for each location showed that in Zelenika 2, *G. pigra* preferred sediment with grain sizes of fraction 2.5 and less of 1.3 (which stays in contrast to the observed patterns in *G. adriatica*) (**Figure 12**). This could be a weak

signal of niche partitioning between the two investigated species. However, based on the data presented in this thesis, a large overlap in niche space is still visible.

One putative explanation of this pattern could be the different size classes (body lengths) sampled for each species. Therefore, only adult individuals larger 40 mm and 45 mm in length were additionally analysed. While the comparison of the relative mass between the individuals larger than 40 mm revealed no statistically significant results and displayed a niche overlap, the dataset for only 45 mm large individuals revealed niche partitioning in the largest fraction (**Figure 15**). Further analyses including correlations of body length and granulometric parameters also indicate two major trajectories for both species (**Figure 17** and **19**). According to this, juvenile (i.e. smaller) *G. adriatica* share similar sorting index and mean granulometric parameters with *G. pigra* (that show almost no gravel size dependency). Hence, ontogenetic shifts in *G. adriatica* from juveniles, living in finer gravels, to adults that inhabit larger stones, can be a likely explanation for this. Also, in other Mediterranean clingfishes, an ontogenetic shift in microhabitat preferences has been previously observed (Goncalves et al. 2002). Above all, this is supported by the overall different size classes sampled for each species. A reason for the overrepresentation of juveniles in *G. adriatica* could be that the sampling method is biased toward finer gravels. Even though the bucket modifications facilitated pulling through the gravel, sampling was physically challenging and sometimes impossible in areas of very coarse gravel and boulders. Further analysis is needed to determine more detailed and distinct microhabitat niche partitioning which should consider sample areas with larger stones like boulders.

Furthermore, in this study, the only variable used to evaluate the species association with benthos was the mass of the sediment and parameters obtained from it. The fact that *Gouania* occupies the space between the rocks was not considered. Nevertheless, it is evident that both species live in the foreshore of the beach composed of gravel and cobbles, which provides them with enough interstitial space to live in. In addition to their stationary benthic lifestyle, they display amphibious emergence behaviour which is mostly likely associated with fluctuations of tidal level (Hofrichter and Patzner 2000; Ord and Cooke 2016; Wagner et al. 2020). Furthermore, *Gouania* probably use sit-and-wait technique to feed on small benthic or planktonic crustaceans, which is exhibited in the clingfish *Gobiesox barbatulus* Starks, 1913 that inhabits similar environments (Pires and Gibran 2011). Because sympatric *Gouania* differ in their morphology, size and microhabitat preferences, it is probable that integration of dietary and environmental factors was an additional driver that allowed ecological

differentiation (Brandl et al. 2020). The macrofauna in the investigated pebble beaches was mainly made up of small amphipods and larger snails (own observation). Hence, it is to be expected that in syntopy different *Gouania* species feed opportunistically on the same food items and do not show a clear preference. This has been observed previously, whereas Mediterranean clingfishes inhabiting rocky environments feed on a variety of prey which is prevailing in the particular environment (Hofrichter 1995; Trkov and Lipej 2019). However, multiple combined approaches in the future will provide a better insight on the ecology of species.

5.2. Geology as a proxy for species occurrence?

The principle component analysis and the relative mass analysis showed that three largest fractions play in part a role in the microhabitat niche partitioning of sympatric *Gouania*, but these associations are strongly correlated with body length. Hence, the size of both species can be correlated with several different granulometric parameters (see above). Accordingly, the size of *G. adriatica* has an inverse connection with the granulometric parameters, mean and sorting. The smaller specimens of *G. adriatica* can be found in moderately sorted sediments and in the sediments with a bigger mean. In comparison, larger *G. adriatica* can be found in very well sorted sediments and in sediments with a smaller mean. To better understand the nature of the granulometric parameters and the size of the species, it is worth mentioning that sorting depends on grain size, but also on the sediment transport mechanics and hydraulic factors (McLean and Kirk 1969). A better sorted sediment can be associated with both sand and gravel as well as sediment transport by water (McLean and Kirk 1969). Gravel tends to move cross-shore, whereas sand usually moves longshore (Ciavola and Castiglione 2009). Moreover, it is worth noting that 97% of the sediment was gravel and the rest was sand, so when discussing “smaller” and “larger” mean, we are talking about the size range of the gravel. Regarding *G. pigra*, an increase in total length was associated with an increase in sediment median, which confirms the remarks made in the field. However, it should be noted that the mesh sizes and the sieves used in this study were not standardised sieves that are used for geological sieve analysis. Therefore, it is not possible to compare these results with granulometric parameters for beaches, obtained from geological sieve analysis.

5.3. Anthropogenic habitat alterations and species threats

On the global scale, coastal zones and their environments are rapidly changing, mainly due to anthropogenic activities such as industry, urbanization and tourism, and global climate changes (Pikelj and Juračić 2013). The eastern Adriatic coast represents one of the most rapidly growing tourist markets in the Mediterranean, with beaches as leading tourism resources (Pikelj and Juračić 2013). Beaches are only 5% of the total length of the eastern Adriatic coast and their small proportion highlights their value in the tourist valorisation (Pikelj and Juračić 2013). The Croatian part of the eastern Adriatic coast is characterised as a drowned karstic coast, with gravel pocket beaches in carbonates or mixed sand gravel beaches formed in flysch rock assemblages (Pikelj and Juračić 2013, 2014). *Gouania* is present only on these types of small and scattered beaches.

Gouania inhabits a highly energetic environment where waves and tides are predominant natural factors, but the growing anthropogenic pressure is changing these dynamics (Wagner et al. 2019). In autumn, when sampling occurred, there were still some swimmers and sunbathers present, as well as in spring when I examined the beaches for presence of *Gouania*. This shows that these beaches are not only used during the “tourist season”, but throughout the whole year. Furthermore, in late spring 2020, excavators were seen transferring the gravel from the backshore to the foreshore of Ambrela Beach. This beach is one of the most visited beaches in Pula and home to *G. pigra*, which was not found there in the next few days after this event had taken place. What happened to the piglet suckers is unknown. Perhaps they moved deeper inshore, as they presumably do during low tide (own observation). In addition, beach nourishment poses a great threat to both species, most likely greater than gravel translocation mentioned before. Given that these species live in the interstitial space of gravel beaches, adding sand will result in filling this interstitial space and it will probably have lethal consequences for the endemic *G. adriatica*, and *G. pigra* (Naqvi and Pullen 1982; Speybroeck et al. 2006; Carević 2020). Even if they managed to escape the massive amount of sand that is pumped into the beach, the loss of prey and a suitable habitat for re-colonisation would present additional threats (Speybroeck et al. 2006). The effect of beach modification on these species is yet to be studied, but there is no doubt that it will have a negative impact. The habitat of *G. willdenowi*, whose range has changed to the western part of the Mediterranean exclusively, and the biocenosis of infralittoral gravels are protected by *Regulation of habitat types, habitat maps, threatened and*

rare habitat types and measures for the conservation of habitat types (Narodne novine 88/2014). Therefore, the habitat of the piglet sucker and the Adriatic blunt-snouted clingfish is protected as well and should be retained in a favourable state of conservation with minimal further degradation.

6. CONCLUSION

Based on the results obtained in this study from the comparison of the total body lengths, the relative masses of the sediments and the sieve analysis, I can conclude that:

- The niches of *G. adriatica* and *G. pigra* mostly overlap, but with an increase in body size, the species showcase a niche partitioning in the largest sediment fractions.
- The size of the species plays a great role in choosing a suitable microhabitat, along with several different granulometric parameters.
- Preferred habitats of both species are dominantly gravel beaches with low amount of sand.
- The sampling method showed to be inadequate in some cases and it should be improved.

The results gained from this study can be used as a guideline for future research of these two species. Better knowledge about the microhabitat niche partitioning and habitat preferences of the sympatric occurring *Gouania* species will help with their possible future protection and monitoring, highlighting the importance of conservation of suitable habitats for only strictly Adriatic endemic fish species, *G. pigra*.

7. LITERATURE

- Ahmadia, G. N., Tornabene, L., Smith, D. J., Pezold, F. L. 2018. The relative importance of regional, local, and evolutionary factors structuring cryptobenthic coral-reef assemblages. *Coral Reefs*, 37, 279– 293.
- Blott, S.J. Pye, K. 2001. GRADISTAT: a grain size distribution and statistics package for the analysis of unconsolidated sediments. *Earth Surface Processes and Landforms* 26, 1237-1248.
- Brandl, S. J., Casey, J. M., Meyer, C. P. 2020. Dietary and habitat niche partitioning in congeneric cryptobenthic reef fish species. *Coral Reefs*, 39(2), 305-317.
- Brandl, S., Bellwood, D. 2014. Individual-based analyses reveal limited functional overlap in a coral reef fish community. *Journal of Animal Ecology*, 83(3), 661-670.
- Brandl, S.J., Goatley, C.H.R., Bellwood, D.R., Tornabene, L. 2018. The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs. *Biol. Rev.* 93, 1846–1873.
- Briggs, J. C. 1955. A monograph of the clingfishes (Order Xenopterygii). Stanford, CA: Stanford Ichthyological Bulletin.
- Carević, D. 2020. Održiva gradnja nasutih plaža Beachex 2019-2023. *Građevinar*, 72(12), 1089-1210.
- Ciavola, P., Castiglione, E. 2009. Sediment dynamics of mixed sand and gravel beaches at short time-scales. *Journal of Coastal Research*, 1751-1755.
- Coleman, R. M. 1999. Parental care in intertidal fishes. *Intertidal fishes: life in two worlds*, 165-180.
- Depczynski, M., Bellwood, D. R. 2003. The role of cryptobenthic reef fishes in coral reef trophodynamics. *Marine Ecology Progress Series*, 256, 183– 191.
- Dirnwöber, M., Herler, J. 2007. Microhabitat specialisation and ecological consequences for coral gobies of the genus *Gobiodon* in the Gulf of Aqaba, northern Red Sea. *Marine Ecology Progress Series*, 342, 265-275.
- Dishman, K. L. 2006. Sieving in particle size analysis. *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*.
- Fricke, R., Eschmeyer, W. N., Fong, J. D. 2020b. Eschmeyer's catalog of fishes: species by family/subfamily. Available at: <http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp>
- Fricke, R., Eschmeyer, W. N., Van der Laan, R. (eds) 2020a. Eschmeyer's catalog of fishes: genera, species, references. Available at: <http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>

- Goatley, C.H., González-Cabello, A., Bellwood, D. 2016. Reef-scale partitioning of cryptobenthic fish assemblages across the Great Barrier Reef, Australia. *Marine Ecology Progress Series*, 544, 271-280.
- Gonçalves, E. J., Barbosa, M., Cabral, H. N., Henriques, M. 2002. Ontogenetic shifts in patterns of microhabitat utilization in the small-headed clingfish, *Apletodon dentatus* (Gobiesocidae). *Environmental Biology of Fishes*, 63(3), 333-339.
- Hofrichter, R. 1995. Taxonomie, Verbreitung und Ökologie von Schildfischen der Unterfamilie Lepadogastrinae (Gobiesocidae, Teleostei). Ph. D. Thesis, University of Salzburg, Salzburg. 469 pp.
- Hofrichter, R., Patzner, R. 2000. Habitat and Microhabitat of Mediterranean Clingfishes (Teleostei: Gobiesociformes: Gobiesocidae). *Marine Ecology*, 21, 41-53.
- Kovačić, M. 1997. Cryptobenthic gobies (Pisces, Perciformes, Gobiidae) and clingfishes (Pisces, Gobiesociformes, Gobiesocidae) in the Kvarner area, Adriatic Sea. *Natura Croatica: Periodicum Musei Historiae Naturalis Croatici*, 6(4), 423-435.
- Kovačić, M., Patzner, R. A., Schliewen, U. 2012. A first quantitative assessment of the ecology of cryptobenthic fishes in the Mediterranean Sea. *Marine Biology*, 159(12), 2731-2742.
- La Mesa, G., Di Muccio, S., Vacchi, M. 2006. Structure of a Mediterranean cryptobenthic fish community and its relationships with habitat characteristics. *Marine Biology*, 149(2), 149-167.
- Magaš, N. 1968. Osnovna geološka karta SFRJ 1:100 000, list Cres L33-113. Institut za geološka istraživanja, Zagreb (1965), Savezni geološki zavod, Beograd.
- McLean, R. F., Kirk, R. M. 1969. Relationships between grain size, size-sorting, and foreshore slope on mixed sand-shingle beaches. *New Zealand Journal of Geology and Geophysics*, 12(1), 138-155.
- Naqvi, S. M., Pullen, E. J. 1982. Effects of beach nourishment and borrowing on marine organisms. US Army Corps of Engineers: Fort Belvoir, VA, Coastal Engineering Research Center.
- Ord, T. J., Cooke, G. M. 2016. Repeated evolution of amphibious behavior in fish and its implications for the colonization of novel environments. *Evolution*, 70(8), 1747-1759.
- Pikelj, K., Juračić, M. 2013. Eastern Adriatic Coast (EAC): geomorphology and coastal vulnerability of a karstic coast. *Journal of coastal research*, 29(4), 944-957.
- Pikelj, K., Juračić, M. 2014. Coastal typology and related seabed sediments along the Eastern Adriatic coast (Croatia). In 5th International Symposium Monitoring of Mediterranean coastal areas: problems and measurement techniques-Abstracts, Livorno, Italy, 17-19 June, 2014. 175-176.

Pires, T. H., Gibran, F. Z. 2011. Intertidal life: field observations on the clingfish *Gobiesox barbatulus* in southeastern Brazil. *Neotropical Ichthyology*, 9(1), 233-240.

Polšak, A. 1967: Osnovna geološka karta SFRJ 1:100 000, list Pula L33-112. Institut za geološka istraživanja, Zagreb (1963), Savezni geološki zavod, Beograd.

Pravilniku o popisu stanišnih tipova, karti staništa te ugroženim i rijetkim stanišnim tipovima. Narodne novine 88/14.

R Core Team. 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Šikić, D., Polšak, A., Magaš, N. 1969: Osnovna geološka karta SFRJ 1:100 000, list Labin L33-101. Institut za geološka istraživanja, Zagreb (1958-1967), Savezni geološki zavod, Beograd.

Speybroeck, J., Bonte, D., Courtens, W., Gheschiere, T., Grootaert, P., Maelfait, J., Mathys, M., Provoost, S., Sabbe, K., Stienen, E., Lancker, V., Vincx, M., Degraer, S. 2006. Beach nourishment: an ecologically sound coastal defence alternative? A review. *Aquatic Conservation-marine and Freshwater Ecosystems*, 16, 419-435.

Tornabene, L., Ahmadi, G. N., Berumen, M. L., Smith, D. J., Jompa, J., Pezold, F. 2013. Evolution of microhabitat association and morphology in a diverse group of cryptobenthic coral reef fishes (Teleostei: Gobiidae: *Eviota*). *Molecular Phylogenetics and Evolution*, 66, 391– 400.

Trkov, D., Lipej, L. 2019. A non-destructive method for assessing the feeding habits of coastal fish. *Mediterranean Marine Science*, 20(2), 453-459.

Wagner, M., Bračun, S., Skofitsch, G., Kovačić, M., Zogaris, S., Iglésias, S. P., Sefc K. M., Koblmüller, S. 2019. Diversification in gravel beaches: a radiation of interstitial clingfish (*Gouania*, Gobiesocidae) in the Mediterranean Sea. *Molecular Phylogenetics and Evolution* 139,106525.

Wagner, M., Kovačić, M., Koblmüller, S. 2020. Unravelling the taxonomy of an interstitial fish radiation: Three new species of *Gouania* (Teleostei: Gobiesocidae) from the Mediterranean Sea and redescriptions of *G. willdenowi* and *G. pigra*. *Journal of Fish Biology*. In press.

Wainwright, D. K., Kleinteich, T., Kleinteich, A., Gorb, S. N., Summers, A. P. (2013). Stick tight: suction adhesion on irregular surfaces in the northern clingfish. *Biology letters*, 9, 20130234.

Yamada, T., Sugiyama, T., Tamaki, N., Kawakita, A., Kato, M. 2009. Adaptive radiation of gobies in the interstitial habitats of gravel beaches accompanied by body elongation and excessive vertebral segmentation. *BMC Evolutionary Biology*, 9, 1– 14.

<https://goo.gl/maps/g6cUKmdFZBsMmNsQ6> (accessed 27.1.2021.)

8. CURRICULUM VITAE

Personal data

Name: Maja Pamić

Date of birth: April 7th, 1996

Nationality: Croatian

Place of birth: Pula, 52100

Place of residence: Vodovodna ulica 8, 52100 Pula

Education

2018 – GRADUATE STUDY – Graduate study of environmental sciences, Faculty of Science, Horvatovac 102a, 10000 Zagreb

2015-2018 – UNDERGRADUATE STUDY – Undergraduate study of environmental sciences, Faculty of Science, Horvatovac 102a, 10000 Zagreb. The title of the thesis: "The endangerment of the Noble Pen Shell (*Pinna nobilis* Linnaeus, 1758) in the Mediterranean Sea".

2011-2015 – SECONDARY SCHOOL – Pula Secondary School, Trierska 8, 52100 Pula

2003-2011 – ELEMENTARY SCHOOL – Šijana Elementary School, 43. istarske divizije 5, 52100 Pula

Work experience

2015 – work through the Student Service for Zigante tartufi d.o.o.

2017 – demonstrations at the Division of Zoology, course Fundamentals of Biology

Professional activity

2018 – participated in the Research and Educational Project "Šuma Žutica 2018".

2018 – participated as an educator at the scientific-popular event "Noć biologije"

2021 – interview on the Adriatic *Gouania* for Austrian show Dimensionen, Ö1 (<https://oe1.orf.at/programm/20210208/627623/Schildfische>)

Volunteer work

2013-2016 – humanitarian work in the Association of Cerebral Palsy of Istria County

Skills

- computer skills (Windows, Office, R, ArcGIS, Past, Primer)
- languages - Italian (passive)
- driver's license for B vehicle category