



## Gastrotrichs and tardigrades in a remnant of Atlantic Forest (Serra do Japi, SP, Brazil)

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GUIDETTI, E.B., CAMPOS, A., BATISTÃO, A.R., SILVA, A.T., BILATTO, C.G., SALGADO, K.A., ARAÚJO, T.Q., GARRAFFONI, A.R.S. **Gastrotrichs and tardigrades in a remnant of Atlantic Forest (Serra do Japi, SP, Brazil)**. Neotropica 21(2): e20201165. <https://doi.org/10.1590/1676-0611-BN-2020-1165>

**Abstract:** Serra do Japi, located in the southeast of São Paulo State, is considered a priority area for conservation, as it houses original Atlantic Forest cover remains. Despite the significant number of studies about vertebrates and invertebrates that were carried out in this region, the meiofauna biodiversity is completely unknown. Thus, the present study aimed to investigate for the first time freshwater Gastrotricha and limnoterrestrial Tardigrada in Serra do Japi Biological Reserve. Samples of sediments, periphyton and floating vegetation in reservoirs and natural lagoons, and mosses growing on native and non-native tree trunks were collected in May 2019. At least five gastrotrichs morphotypes were identified and three of them were formally described: *Chaetonotus acanthocephalus*, *C. dadayi* (first record in Brazil), and *Heterolepidoderma mariae* (first record outside the type locality). In regards to tardigrades, twelve morphotypes were identified and four of them were formally described: *Pseudechiniscus juanita*, *Minibiotus cf. acantistus*, *Echiniscus dreyfusi* and *Itaquascon umbellinae* (last two species reported for the first time outside the type locality). This study reinforces that meiofaunal diversity and distribution have been underestimated, even in one of the five largest hotspots in the world.

**Keywords:** Checklist, meiofauna, floating vegetation, moss.

## Gastrótricos e tardígrados em um remanescente de Mata Atlântica (Serra do Japi, SP, Brasil)

**Resumo:** A Serra do Japi, localizada no sudeste do Estado de São Paulo, é considerada uma área prioritária para conservação, por abrigar vestígios de cobertura original de Mata Atlântica. Apesar do número significativo de estudos sobre vertebrados e invertebrados realizados nesta região, a biodiversidade de grupos meiofaunais é completamente desconhecida. Assim, o presente estudo teve como objetivo investigar pela primeira vez Gastrotricha de água doce e Tardigrada limnoterrestre na Reserva Biológica da Serra do Japi. As amostras de sedimentos, perifiton e vegetação flutuante em reservatórios e lagoas naturais, e musgos presentes em troncos de árvores nativas e não nativas foram coletadas em maio de 2019. Foram encontrados cinco morfotipos de gastrótricos, sendo que apenas três deles já foram formalmente descritos: *Chaetonotus acanthocephalus*, *C. dadayi* (primeiro registro no Brasil) e *Heterolepidoderma mariae* (primeiro registro fora da localidade-tipo). Em relação aos tardígrados, foram identificados doze morfotipos, sendo que quatro deles já formalmente descritos: *Pseudechiniscus juanita*, *Minibiotus cf. acantistus*, *Echiniscus dreyfusi* e *Itaquascon umbellinae* (as duas últimas espécies relatadas pela primeira vez fora da localidade-tipo). Este trabalho reforça que a diversidade e distribuição da meiofauna têm sido subestimadas, mesmo em um dos cinco maiores hotspots do mundo.

**Palavras-chave:** Lista de espécies, meiofauna, vegetação flutuante, musgo.

## Introduction

Meiofauna can be defined as an assembly of freshwater and marine organisms that pass through a coarse sieve of 500  $\mu\text{m}$  and are retained by a finer sieve of 44  $\mu\text{m}$  and do not have close phylogenetic relationships among them (Giere 2009). These animals can be classified as permanent, having an entire life as small organisms, or temporary, having one or more stages of the life cycle with 'meiofaunal' body size (Higgins & Thiel 1988). Beside in the water bodies of rivers and lakes, freshwater meiofauna can be found in distinct environments, such as rooted vegetation, macroalgae fronds, mosses, and some animal structures like worm tubes (Higgins & Thiel 1988, Boeckner et al. 2009, Giere 2009).

Among the 34 phyla of Metazoa, there are at least 23 that have meiofaunal organisms, such as Gastrotricha and Tardigrada (Giere 2009, Cerca et al. 2018). Gastrotricha are microinvertebrates living in marine (e.g. among sand grains and algae) and freshwater (e.g. aquatic plants and psammon) habitats around the world (Balsamo et al. 2014, 2020, Kieneke & Schmidt-Rhaesa 2015). The taxon is divided into two orders: Macrotrichida, with a worm-like body consisting of 380 species, all marine except for four species (Garraffoni et al. 2019a, Balsamo et al. 2020); Chaetonotidae, with a tenpin-shaped body consisting of 480 species, two-thirds of which reported in freshwater (Balsamo et al. 2008, 2014, 2020). Tardigrada, commonly known as water bears, living in marine (e.g. sand grains, rock shore, barnacles), freshwater (e.g. aquatic plants and psammon), and limnoterrestrial (e.g. bryophytes, cushion-forming plants, lichens, algae, soils) habitats (Nelson et al. 2015). The taxon comprises more than 1300 species divided into two valid classes: Heterotardigrada (majority marine); and Eutardigrada (freshwater and limnoterrestrial). The existence of a third class, Apotardigrada (freshwater and limnoterrestrial) has been previously discussed (Guil et al. 2019, Morek et al. 2020).

Due to the meiofaunal body size and challenging identification process, the knowledge about gastrotrichs and tardigrades' diversity in the world is underestimated (Vicente 2010, Appeltans et al. 2012; Balsamo et al. 2014, 2015, 2020, Fonseca et al. 2018, Garraffoni et al. 2019b). Notwithstanding, a few studies have aimed to reveal the richness and distributional patterns of these two freshwater and limnoterrestrial meiofauna taxa in Brazil (de Barros R 1939a, b, 1942a, b, 1943, Kisielewski 1991, Assunção 1999, Pilato et al. 2002b, Garraffoni et al. 2010, 2017a, b, 2019a, Garraffoni & Melchior

2015, Araújo et al. 2013, Rocha et al. 2016, Garraffoni 2017, de Barros RC 2020). This study aims to contribute to this knowledge by presenting the first records of gastrotrichs and tardigrades at the Biological Reserve of Fundação Serra do Japi, an extensive area of Atlantic Forest located in São Paulo State. In addition, this manuscript provides taxonomic notes of the morphotypes and formally described species found at the Reserve.

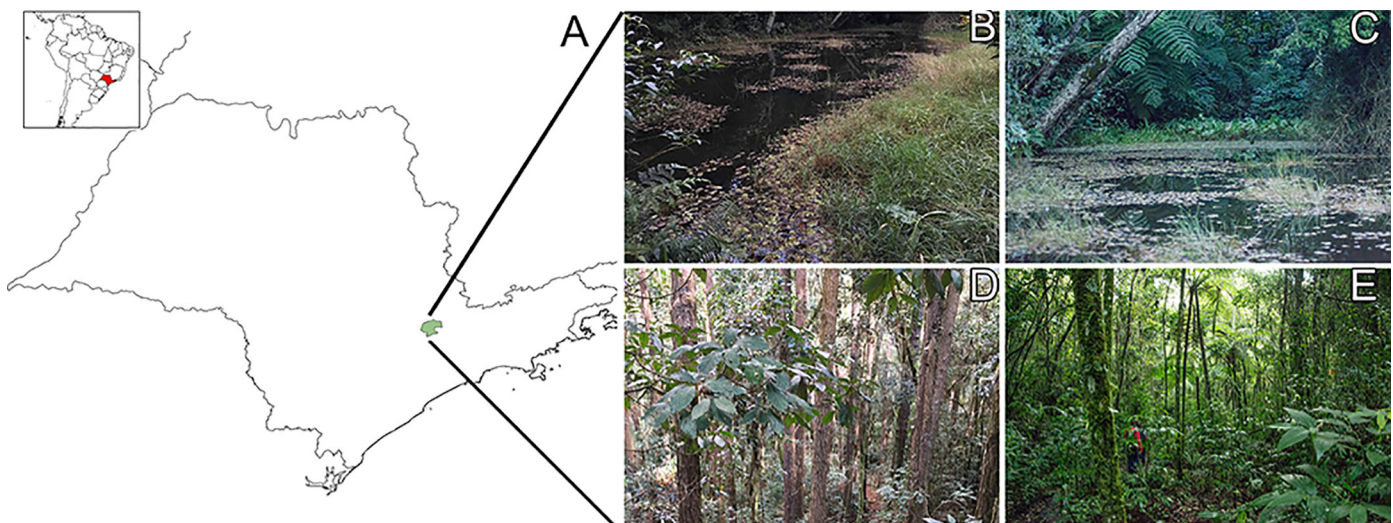
## Material and Methods

### 1. Study site

Serra do Japi is located in the southeast of São Paulo, bordering four cities (Jundiá, Pirapora do Bom Jesus, Cajamar and Cabreúva), between the geographical coordinates 23°14'0"S and 46°58'0"W. The elevation has an area of approximately 35,000 hectares and a native vegetation cover of semi-deciduous mesophyllous forests, semi-deciduous forests of altitude, and sparse rocky enclaves (Morellato 1992) and fragments of reforestation with pine and *Eucalyptus* sp., pastures, and small portions of agricultural crops. The altitudinal ranges vary between 700 and 1,000 meters (Pinto et al. 1972). In the coldest month, July, the average temperatures range between 11.8°C and 15.3°C, and in the warmest month, January, between 18.4°C and 22.2°C depending on the altitude (Morellato 1992). The rainfall cycle is variable, increasing in the months of December-January, with values greater than 250 mm per month, and decreasing in the winter, with values lower than 41 mm and 71 mm per month, depending on the location (Morellato 1992).

### 2. Collecting procedures and Data analysis

Samples were collected from May 18<sup>th</sup> to 19<sup>th</sup>, 2019. Gastrotricha specimens were sampled by collecting specimens of the floating vegetation belonging to *Salvinia* sp. from a small artificial reservoir (23°14'42.8"S, 46°56'12.4"W) and natural lagoon (23°14'47.3"S, 46°56'12.4"W) and stored in plastic buckets. Tardigrada specimens were sampled by collecting mosses belonging to family Pottiaceae on native (23°14'21.9"S, 46°56'07.6"W) and from *Sematophyllum galipense* (Müll.Hal.) Mitt. on exotic *Pinus* sp. (23°13'59.8"S, 46°56'01.5"W) trees (Figure 1) and stored in paper bags. Freshwater and limnoterrestrial samples were brought back to the University of Campinas for further analysis.



**Figure 1.** Map of collection stations. A: Serra do Japi Biological Reserve, B: floating vegetation from an artificial small reservoir, C: natural lagoon, D: exotic *Pinus* sp. tree trunks, E: mosses from the Atlantic Forest.

Water with sediment was sieved (42 µm mesh) and specimens were encountered by sorting small amounts of sediment poured into Petri dishes under a Zeiss Stemi 2000 stereomicroscope. Mosses were placed in a beaker filled with ddH<sub>2</sub>O, and after 20 minutes the water was transferred to Petri dishes and the specimens were scanned under a Zeiss Stemi 2000 stereomicroscope.

Alive specimens were isolated with micropipettes into a glass embryo dish. The gastrotrichs and tardigrades were anesthetized with 2% MgCl<sub>2</sub> and warm water (70°C), respectively. Subsequently, gastrotrichs were isolated and mounted on glass slides and tardigrades were stored in 70% ethyl alcohol and permanent slides were made using Fluoromount Aqueous Mounting medium. All specimens were documented under a Zeiss Axio Imager M2 light microscope equipped with Differential Interference Contrast optics (DIC) connected to a camera. Photomicrographs were taken using the software ZEN - blue edition. The most representative structures of tardigrades identified up to the specific level were measured. Methods and terminology used to measure the specimens of *Echiniscus dreyfusi* followed Bartylak et al. (2019); *Pseudechiniscus juanita* followed Roszkowska et al. (2020); *Itaquiscon umbellinae* followed Pilato et al. (2002a), *Minibiotus* cf. *acontistus* followed Kaczmarek & Michalczyk 2017. Morphometric data was handled using “Echiniscoidea” and “Parachela” templates available from the Tardigrada Register (Michalczyk & Kaczmarek 2013).

Photos of Gastrotricha (due to the fragility of their bodies, they were destroyed during the microscopic examination and are no longer available - Balsamo et al. 2014, 2020, Garraffoni et al. 2019b) and photos and permanent slides of Tardigrada specimens are available at the Zoological Museum “Adão José Cardoso” (ZUEC), at the University of Campinas, Brazil.

## Results

At least five morphospecies (38 specimens) of Gastrotricha belonging to two genera of Chaetonotidae were present. Among these five morphospecies, three were attributed to described species (*Chaetonotus dadayi* Schwank, 1990, *Chaetonotus acanthocephalus* Valkanov, 1937 and *Heterolepidoderma mariae* Garraffoni & Melchior, 2015). A total of 12 morphospecies (32 specimens) belonging to two classes of Tardigrada were present. Among these 12 morphospecies, eight were identified at the genus level and four were attributed to described species (*Itaquiscon umbellinae* de Barros R, 1939a, *Pseudechiniscus juanita* de Barros R, 1939b, *Minibiotus* cf. *acontistus* de Barros R, 1942b and *Echiniscus dreyfusi* de Barros R, 1942a). The remaining morphospecies could not be described to species level due to problems in the fixation process (see Discussion).

### Phylum Gastrotricha

Order Chaetonotida Remane, 1925 [Rao & Clausen, 1970]

Suborder Paucitubulatina d’Hondt, 1971

Family Chaetonotidae Gosse, 1864 [Garraffoni, Araújo, Lourenço, Guidi & Balsamo, 2017b]

Subfamily Chaetonotinae Kisielewski, 1991

Genus *Chaetonotus* Ehrenberg, 1830

### *Chaetonotus (Primochaetus) acanthocephalus* Valkanov, 1937

Figure 2 (A-C)

**Examined material:** One specimen (ZUEC PIC 390).

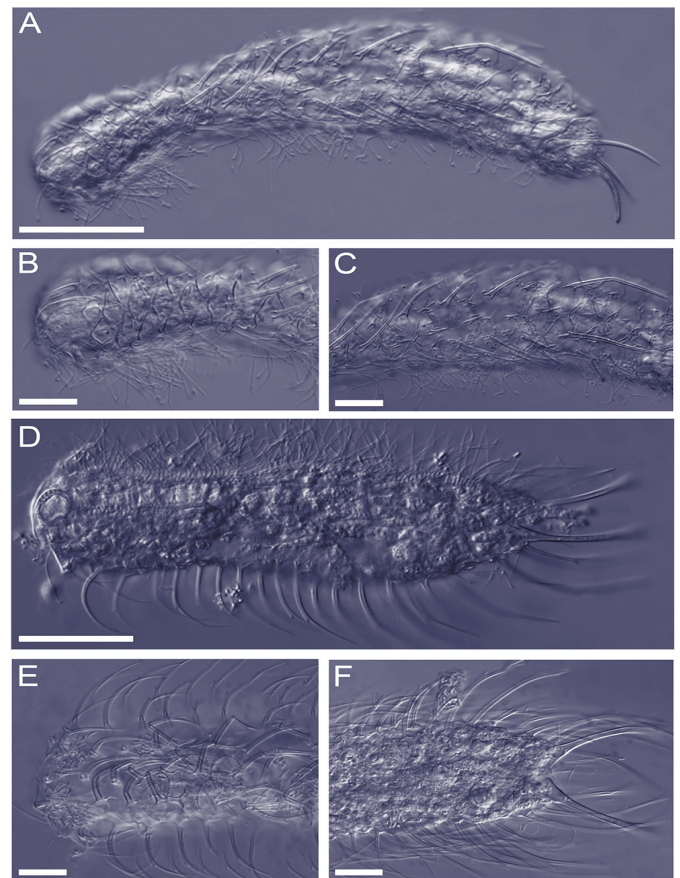
**Remarks:** *C. acanthocephalus* is easily recognized by the presence of five dorsal cephalic scales with long spines, two plates at the side of the hypostomium, and the arrangement of dorsal and lateral spines (e.g. two pairs at the neck, transversal row at the mid-dorsal surface). This species was previously reported in Poland, Germany, Romania, Bulgari (Valkanov 1937, Kisielewski 1981, Schwank 1990) and Brazil (Kisielewski 1991, Garraffoni et al. 2010, Araújo et al. 2013).

### *Chaetonotus (Chaetonotus) dadayi* Schwank, 1990

Figure 2 (D-F)

**Examined material:** Fourteen specimens (ZUEC PIC 377-389).

**Remarks:** Von Daday (1905) identified specimens found on the border between Paraguay and Brazil, in Estia Postillon, as *Chaetonotus similis* Zelinka, 1889. Later, Schwank (1990) recognized that the specimens analyzed by Von Daday (1905) had a pair of spines that protruded beyond the adhesive tubes. This feature is not observed in *Chaetonotus similis* and Schwank (1990) described a new species, *C. dadayi*, to accommodate the Paraguayan organisms. Several years later, d’Hondt (2006) found *C. dadayi* in French Guiana and presented the first photographic record of a specimen of this species. The Brazilian specimens are morphologically close to the previous descriptions due to the presence of spined scales of the posterior dorsal region smaller than the trunk ones, lateral spined scales of the furca base longer than adhesive tubes; two pairs of long, thick, and simple spines on the dorsal



**Figure 2.** DIC photomicrograph of Gastrotricha. A-C: Lateral view of *Chaetonotus (Primochaetus) acanthocephalus*, D, F: Ventral view of *Chaetonotus (Chaetonotus) dadayi*, E: Dorsal view of *Chaetonotus (Chaetonotus) dadayi*. Scale bars: 50 µm.

region and two pairs of scales with long, thin and simple spines in the ventral region. This is the first report from Brazil.

### *Chaetonotus* sp. 1

Figure 3 (A-C)

**Examined material:** Four specimens.

**Remarks:** Mouth terminal; pentalobate head. Trilobate spined scales on dorsal and ventrolateral regions; spines increasing in length along the body; set of nine long and thick spines arranged as 3-3-3 on the middle dorsal region. Ventral interciliary portion covered by small spined scales.

### *Chaetonotus* sp. 2

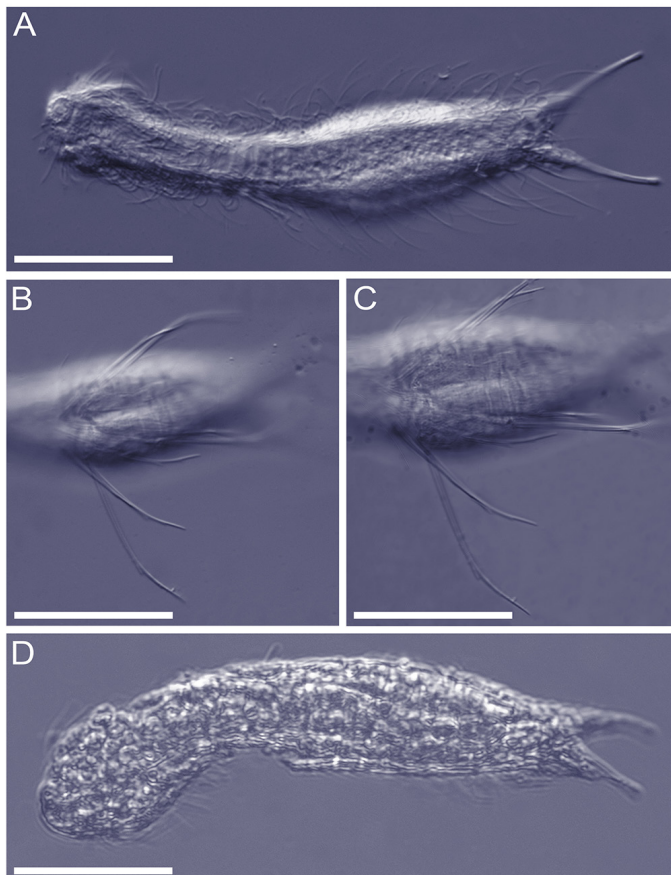
**Examined material:** Six specimens.

**Remarks:** Mouth subterminal; small cephalic hypostomium. Rounded spined double-edge scales on dorsal and ventrolateral regions; dorsal spines increasing in length from head to middle trunk and decreasing until the posterior end; pair of long spines observed at the furcal base. Ventral interciliary portion covered by small rounded spined scales.

Genus *Heterolepidoderma* Remane, 1927

### *Heterolepidoderma mariae* Garraffoni & Melchior, 2015

Figure 3 (D)



**Figure 3.** DIC photomicrograph of Gastrotricha. A: Ventral view of *Chaetonotus* sp. 1, B-C: Dorsal view of *Chaetonotus* sp. 1, D: Ventral view of *Heterolepidoderma mariae*. Scale bars: 50  $\mu$ m.

**Examined material:** Six specimens (ZUEC PIC 391-396).

**Remarks:** *Heterolepidoderma mariae* can be easily recognized by specific cuticular ornamentation of the dorsal body surface, keeled scales with three different shapes. It is the first report outside the type locality, Paulínia - São Paulo State, Brazil (Garraffoni & Melchior 2015).

### Phylum Tardigrada

Class Heterotardigrada Marcus, 1927

Order Echiniscoidea Richters, 1926

Family Echiniscidae Thulin, 1928

Genus *Echiniscus* Schultze, 1840

### *Echiniscus dreyfusi* de Barros R, 1942

Figure 4 (A), Table 1

**Examined material:** One specimen, unidentified sex, (ZUEC PIC 402).

**Remarks:** The specimen found in Serra do Japi has four features not mentioned in the original description of *Echiniscus dreyfusi*: thick granulation on the dorsal side of the legs; spine present on the first pair of legs; spurs on claws of the third and fourth pairs of legs; the dorsal pair of spines has a striated texture. This species was previously reported in New Guinea (Iharos 1963), Argentina (Claps & Rossi 1988), Japan (Utsugi 1988), and Brazil (de Barros R 1942a). In Brazil, it is the first report outside the type locality, São Vicente City - São Paulo State.

### *Echiniscus* sp.

Figure 4 (B)

**Examined material:** One specimen, unidentified sex.

**Remarks:** Body orange and plump. Dorsal plates with large pores irregularly distributed; *spinulosus* type. Trunk appendages in the form of spines; spines D<sup>d</sup> and E more robust and rough. There is a small denticle on each D<sup>d</sup> spine. Small spine on the first leg pair. Papilla and dentate collar present on the fourth leg pair.

Genus *Pseudechiniscus* Thulin, 1911

### *Pseudechiniscus juanita* de Barros R, 1939

Figure 4 (C), Table 2

**Examined material:** Three specimens, unidentified sex, (ZUEC PIC 399-401).

**Remarks:** Body orange. Round black eyes present in two specimens. *Cirrus internus*, *externus* and A present. Clava visible in one specimen. Pseudosegmental plate present. Very visible cuticular granulation on the dorsal plates and legs. Papilla on the fourth pair of legs visible in one specimen. Dentate collar absent. Spurs on claws IV present. Spurs on claws I visible in one specimen. This species was previously reported in Austria (Mihelcic 1962, Maucci 1974), Italy (Ramazzotti & Maucci 1983), Galápagos Islands (Schuster & Grigarick 1966), China (Wang 2009), and Brazil (de Barros R 1939b, 1942a, du Bois-Reymond Marcus 1944).

Class Eutardigrada Richters, 1926

Order Parachaela Schuster, Nelson, Grigarick & Christenberry, 1980

Family Macrobiotidae Thulin, 1928

Genus *Paramacrobotus* Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009

### *Paramacrobotus* sp. 1

Figure 4 (D)

**Examined material:** Three specimens, unidentified sex.

**Remarks:** Eyes present in only one specimen. Cuticle smooth. Oral cavity armature (OCA) composed of three bands of teeth. Pharyngeal apophysis present and very visible. Three macroplacoids present and separated by the same distance from each other; the second macroplacoid is the smaller; the third macroplacoid has a small constriction; very small triangular microplacoids present. Claws of the *hufelandi* type.

### *Paramacrobotus* sp. 2

Figure 4 (E)

**Examined material:** Four specimens, unidentified sex.

**Remarks:** Eyes present in two specimens. Cuticle smooth except for very visible granulation present on all legs. Oral cavity armature composed of three bands of teeth. Pharyngeal apophysis present; three macroplacoids present; the second is the smaller and the third is positioned more distant from the others; the third macroplacoid has a small constriction; microplacoids absent. Claws of the *hufelandi* type. Dentate lunules under claws IV.

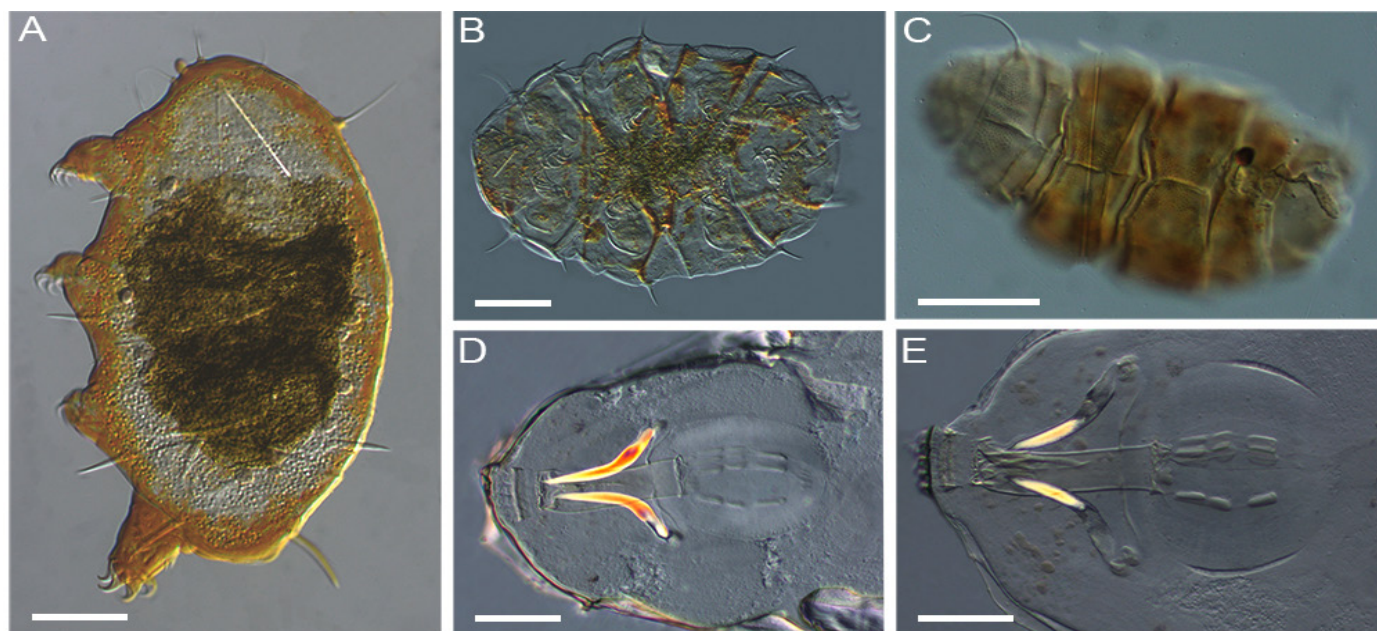
### *Paramacrobotus* sp. 3

**Examined material:** Two specimens, unidentified sex.

**Remarks:** Eyes absent. Cuticle smooth except for very visible granulation present on all legs. Oral cavity armature (OCA) composed of three bands of teeth. Pharyngeal apophysis present. Three macroplacoids present; the second is the smaller and the third is positioned more distant from the others; the third macroplacoid has a small constriction; very

**Table 1.** Measurements [in  $\mu\text{m}$ ] and *sc* values of selected morphological structures of *Echiniscus dreyfusi* de Barros R, 1942. The *sc* is the percentage of scapular plate length (Fontoura et al. 2008).

CHARACTER	$\mu\text{m}$	<i>sc</i>
Body length	279	575
Scapular plate length	48.5	–
Head appendages lengths		
Cirrus internus	20.5	41.2
Cephalic papilla	8.3	17.1
Cirrus externus	20.0	41.2
Clava	8.5	17.5
Cirrus A	40.8	84.1
Cirrus A/Body length ratio	15%	–
Cirrus int/ext length ratio	100%	–
Body appendages lengths		
Cirrus B	20.5	42.3
Cirrus C	34.5	71.1
Cirrus D	32.0	66.0
Cirrus Dd	30.0	61.9
Cirrus E	37.2	76.6
Spine on leg I length	5.4	11.1
Claw 1 heights		
Branch	13.2	27.2
Claw 2 heights		
Branch	12.5	25.8
Claw 3 heights		
Branch	11.7	24.1
Spur	4.1	8.5
Spur/branch height ratio	35%	–
Claw 4 heights		
Branch	16.3	33.6
Spur	4.3	8.9
Spur/branch height ratio	26%	–



**Figure 4.** DIC photomicrograph of Tardigrada. A: Dorsal view of *Echiniscus dreyfusi*, B: Ventral view of *Echiniscus* sp. C: Dorsal view of *Pseudechiniscus juanita*, D: Buccal apparatus of *Paramacrobotus* sp. 1, E: Buccal apparatus of *Paramacrobotus* sp. 2. Scale bars: 50  $\mu\text{m}$ .

**Table 2.** Measurements [in  $\mu\text{m}$ ] and *sc* values of selected morphological structures of *Pseudechiniscus juanitae* de Barros R, 1939b. The *sc* is the percentage of scapular plate length (Fontoura et al. 2008).

CHARACTER	$\mu\text{m}$	<i>sc</i>	$\mu\text{m}$	<i>sc</i>	$\mu\text{m}$	<i>sc</i>
Body length	263	793	181	755	301	564
Scapular plate length	33.2	–	24.1	–	53.4	–
Head appendages lengths						
Cirrus internus	19.3	58.0			13.4	25.1
Cephalic papilla						
Cirrus externus	37.8	113.8			28.8	53.9
Clava	7.4	22.3				
Cirrus A			32.3	134.2	40.1	75.0
Cirrus A/Body length ratio		–	18%	–	13%	–
Cirrus int/ext length ratio	51%	–		–	46%	–
Papilla on leg IV length			5.4	22.6		
Claw 1 heights						
Branch			10.0	41.5	11.2	21.0
Spur					3.1	5.7
Spur/branch height ratio		–		–	27%	–
Claw 2 heights						
Branch			8.4	34.7		
Spur						
Spur/branch height ratio		–		–		–
Claw 4 heights						
Branch	11.2	33.7	9.2	38.2		
Spur	3.0	9.0	3.7	15.5		
Spur/branch height ratio	27%	–	41%	–		–

small triangular microplacoids present. Claws of the *hufelandi* type. Dentate lunules under claws IV.

Genus *Macrobotus* Schultze, 1834

#### *Macrobotus* sp. 1

Figure 5 (A)

**Examined material:** Four specimens, unidentified sex.

**Remarks:** Eyes absent. Dorsal cuticle with very visible ornamentation forming polygons like scales of reptiles; thin granulation present on all legs. Bucco-pharyngeal apparatus of the *Macrobotus* type. Pharyngeal apophysis present; three short and wide macroplacoids present; large microplacoids present. Claws of the *hufelandi* type. Cuticular bars on legs I-III.

#### *Macrobotus* sp. 2

**Examined material:** Two specimens, unidentified sex.

**Remarks:** Eyes present. Cuticle smooth. Pharyngeal apophysis presents; three slender macroplacoids present, the third being positioned more distant from the others. Claws of the *hufelandi* type.

Genus *Minibiotus* Schuster, 1980

#### *Minibiotus* cf. *acotistus* de Barros R, 1942b

Table 3

**Examined material:** One specimen, unidentified sex (ZUEC PIC 398).

**Remarks:** Eyes absent. Cuticle smooth. Buccal tube long and narrow. Three macroplacoids present; microplacoids absent. Claws of the *hufelandi* type (claws Y-shaped; symmetrically arranged with respect to the median plane of the leg; without basal spurs).

#### *Minibiotus* sp.

Figure 5 (B)

**Examined material:** Eight specimens, unidentified sex.

**Remarks:** Eyes present only in two specimens. Cuticle smooth. Buccal tube long and narrow. Large pharyngeal apophyses present. Three macroplacoids present; microplacoids present and very small. Claws of the *hufelandi* type.

Family Hypsibiidae Pilato, 1969

Subfamily Itaquasconinae Rudescu, 1964

Genus *Itaquascon* de Barros R, 1939a

#### *Itaquascon* *umbellinae* de Barros R, 1939a

Figure 5 (C), Table 4

**Examined material:** One specimen, unidentified sex (ZUEC PIC 397).

**Table 3.** Measurements [in  $\mu\text{m}$ ] and *pt* values of selected morphological structures of *Minibiotus cf. acontistus* de Barros R, 1942b. The *pt* index is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato 1981).

CHARACTER	$\mu\text{m}$	<i>pt</i>
Body length	180	662
Buccal tube length	27.2	–
Pharyngeal tube length	6.8	25.0
Buccopharyngeal tube length	34.0	125.0
Buccal/pharyngeal tube length ratio	400%	–
Stylet support insertion point	25.0	91.9
Buccal tube external width	2.3	8.5
Buccal tube internal width	1.3	4.6
Placoid lengths		
Macroplacoid 1	2.0	7.2
Macroplacoid 2	2.8	10.1
Macroplacoid 3	2.3	8.4
Macroplacoid row	12.2	44.9
Claw 1 heights		
External primary branch	4.9	18.0
External secondary branch	4.7	17.3
Internal primary branch	5.4	19.9
Internal secondary branch	4.8	17.6
Claw 2 heights		
External primary branch	5.8	21.3
External secondary branch	5.1	18.8
Internal primary branch	6.2	22.8
Internal secondary branch	6.0	22.1
Claw 3 heights		
External primary branch	6.7	24.6
External secondary branch	5.0	18.4
Internal primary branch	5.3	19.5
Internal secondary branch	4.3	15.8
Claw 4 heights		
Anterior primary branch	6.8	24.8
Anterior secondary branch	5.6	20.4
Posterior primary branch	8.0	29.4
Posterior secondary branch	7.2	26.5

**Remarks:** Eyes absent. Cuticle smooth. Buccal tube long and narrow; pharyngeal bulb oval without placoids. Claws of the *Hypsibius* type (the secondary branch and the basal section form a continuous curve; claws of the same leg are different in size and shape). This species was described from Itaquaquecetuba, São Paulo State, Brazil (de Barros R 1939a), and reported in Argentina (Maucci 1988). In Brazil, this is the first report outside the type locality.

Genus *Adropion* Pilato, 1987

*Adropion* sp.

Figure 5 (D)

**Examined material:** One specimen, unidentified sex.

**Remarks:** Eyes absent. Cuticle smooth. Cuticular thickening between the buccal tube (rigid) and the pharyngeal tube (flexible) absent. Pharyngeal apophyses are small. Three macroplacoids present; microplacoids present. Claws of the *Hypsibius* type.

## Discussion

Meiofauna organisms are represented in  $\frac{2}{3}$  of all known metazoan taxa (Giere 2009, Cerca et al. 2018) and it is impossible to apply a unique methodological protocol to perform fixation and preparation for all meiofaunal taxa (Fonseca et al. 2018). As a consequence, a large amount of these tiny animals, that have small, fragile bodies, remains poorly studied by researchers since the process of sorting and identification is intensive and time sensitive (Appeltans et al. 2012, Fonseca et al. 2018, Garraffoni et al. 2019b, Balsamo et al. 2020).

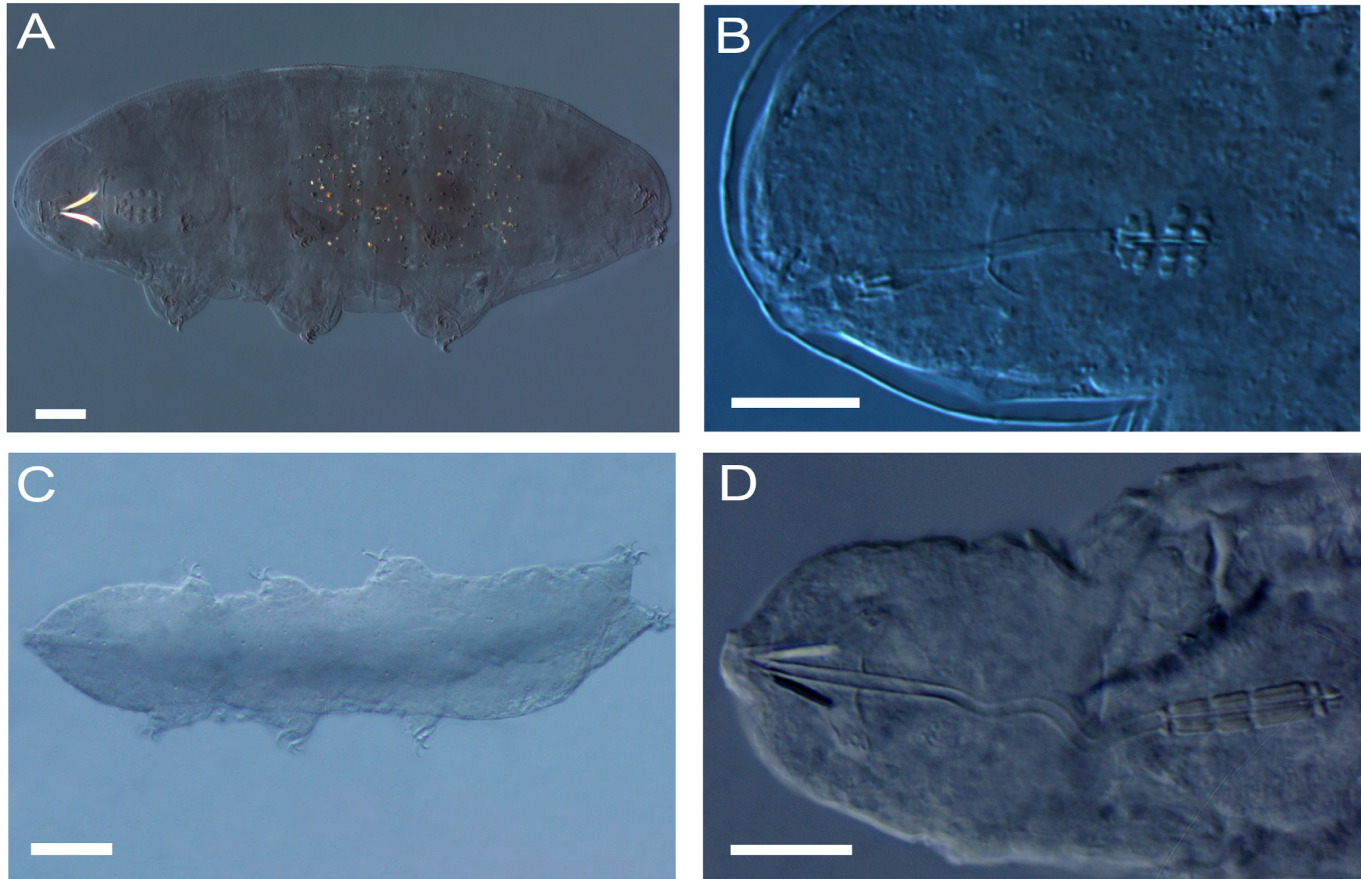
The examination of living microscopic organisms requires patience from the taxonomists, especially those that are beginners in meiofaunal identification. The small size and transparency of the body of meiofaunal individuals make them difficult to be discovered among sediments and for subsequent steps such as collecting them by pipette and isolating them for further studying (Giere 2009, Balsamo et al. 2014, 2020, Nelson et al. 2015). Furthermore, gastrotrichs must be studied alive and are frequently destroyed or even lost during the process of preparation for observations under light microscopy (Balsamo et al. 2014, 2020, Garraffoni et al. 2019b).

If the specimens are not properly fixed, they can easily vanish or not stay in a suitable position for future observations of the morphological features (Nelson et al. 2015, Fonseca et al. 2018, Garraffoni et al. 2019b, Balsamo et al. 2014, 2020). In these cases, due to losses of important morphological information, the accuracy of the species identification decreases, and misidentifications are inevitable (Schill 2018). Even in tardigrades that have an exocuticle that remains preserved after the fixation process, morphological information of taxonomic importance can be lost (Morek et al. 2016).

This study is a direct result of undergraduate lectures that aimed to teach not only meiofaunal diversity but also how to collect, sort, and identify these tiny animals, using gastrotrichs and tardigrades as study models. Thus, all the problems described above were considered and largely overcome by the students during the lectures. As a result, we found at least five morphospecies of Gastrotricha and 12 of Tardigrada, but within these 17 morphospecies, only seven of them (41,2%) were identified at a specific level. It is important to highlight that we could not find more morphospecies of Gastrotricha because many specimens were poorly fixed or deteriorated, preventing proper identification beyond the genus level.

Regarding the gastrotrichs, two of the three identified species and all unidentified morphotypes belong to the most speciose genus in the taxon, *Chaetonotus* Ehrenberg, 1830 with more than 250 species formally described (Balsamo et al. 2020). The species *Chaetonotus dadayi* was found in Brazil for the first time and these samples will be important to provide material for a near future redescription of *this species* providing detailed morphological and ecological data.

The morphotype *Chaetonotus* sp. 1 has a remarkable feature of nine long spines arranged in three subsequent groups of three spines in the middle of the trunk on the dorsal surface. These conspicuous spines in the middle of the trunk are found in other representatives of this taxon and can be arranged in a “belt”, in a subsequent transverse row, or inserted in large nearby scales forming a grouping (Araújo et al.



**Figure 5.** DIC photomicrograph of Tardigrada. A: Dorsal view of *Macrobiotus* sp. 1. B: Buccal apparatus of *Minibiotus* sp. C: Ventral view of *Itaquascon umbellinae*. D: Buccal apparatus of *Adropion* sp. Scale bars: 50  $\mu\text{m}$ .

**Table 4.** Measurements [in  $\mu\text{m}$ ] of selected morphological structures of *Itaquascon umbellinae* de Barros R, 1939a.

CHARACTER	$\mu\text{m}$
Body length	243
Buccal tube external width	3.9
Buccal tube internal width	3.3
Claw 1 heights	
External	9.0
External primary branch	6.7
External secondary branch	5.0
Internal	6.1
Claw 2 heights	
External	10.6
External primary branch	7.5
External secondary branch	5.4
Internal	5.9
Claw 3 heights	
External	9.9
External primary branch	7.2
External secondary branch	4.5
Internal	5.8
Claw 4 heights	
External	11.4
External primary branch	8.8
External secondary branch	6.3
Internal	6.4

2013). Kisielewski (1991) reported two species of *Chaetonotus* in Brazil with long spines on the dorsal region, *C. bisacer* and *C. succinctus*. Representatives of these species have spines arranged in a “belt” shape, differing from *C. sp.1*, which presents three spines closely inserted in three subsequent groups of spines. Araújo et al. (2013) also reported an unidentified species of *Chaetonotus* with long spines on the dorsal region, but with five and not nine as described for the present one.

Due to the shape and distribution of the dorsal and ventral scales and a pair of spines over the furcal base, the morphotype *Chaetonotus* sp. 2 is similar to two species reported for Brazil by Kisielewski (1991), *C. intermedius* and *C. breviacanthus*. However, *C. sp. 2* differs from both species by the presence of a double-edge scale and by the pair of long spines at the furcal base. In comparison to *C. intermedius*, it was not possible to visualize the two pairs of ventral terminal scales and the cuticular pharyngeal rod. Additionally, in comparison to *C. breviacanthus*, conspicuous protuberance at the anterior edges of the hypostomium and the posterior “U” edge of the scales were not observed in any specimens of *C. sp.2*.

Despite the fact that tardigrades have a rigid and armored integument and morphological investigations of living individuals are not required, from the 12 morphotypes of Tardigrada found in the present study, eight were not identified to species level. This occurred because in many species the study of adult morphology alone is not enough for species identification (Nelson et al. 2015, Schill 2018). Very often it is necessary to analyze the eggs because they have essential taxonomic significance



and show species-specific ornamentation. In particular, within the taxon Macrobiotidae, limnic and terrestrial tardigrades belonging to the genera *Minibiotus* Schuster, 1980, *Macrobious* Schultze, 1834 and *Paramacrobious* Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009 (see Dastych 1998, Meyer & Hinton 2009, Kaczmarek et al. 2017), can only be properly identified if the eggs are available.

The genus *Minibiotus* has 50 described species morphologically diverse (Degma et al. 2020). According to recent studies, the results obtained with the use of integrative approach using genetic and detailed morphological data, e.g. in the description of the species *Minibiotus ioculator* Stec, Kristensen and Michalczyk 2020a, can clarify the species relationships and contribute with information for status polyphyletic of the group (Stec et al. 2020a).

The only four species of *Minibiotus* genus reported in Brazil were: *Minibiotus acontistus* (de Barros R 1942), *Minibiotus intermedius* (Plate 1888), *Minibiotus julietae* (de Barros R 1942) and *Minibiotus marcusii* (de Barros R 1942) (de Barros R 1942b, du Bois-Reymond Marcus 1944, Kaczmarek et al. 2015). Two *Minibiotus* morphospecies were sampled in the present study and one of them was very similar to *M. acontistus* described by de Barros R (1942b). Among the genus *Macrobious*, one of the most speciose groups within Tardigrada, we were only able to highlight that *Macrobious* sp. 1 shows a very distinct dorsal cuticle ornamentation.

Three morphospecies of the genus *Paramacrobious* were found: *Paramacrobious* sp. 1, *Paramacrobious* sp. 2 e *Paramacrobious* sp. 3. Despite that species belonging to this genus have a very restricted biogeographic distribution range (Kaczmarek et al. 2017), for an accurate description of any *Paramacrobious* species it is important to use an integrative taxonomy approach (Stec et al. 2020b). Furthermore, recently the subgeneric classification was revisited and nomenclature acts and diagnoses were changed (Marley et al. 2018).

The genus *Adropion* Pilato, 1987 is currently composed of 15 species (Degma et al. 2020), and considered a polyphyletic group, represented by three independent evolutionary lineages (Gasiorek & Michalczyk 2020). The unique species reported in Brazil was *Adropion scoticum* Murray, 1905 (de Barros R 1943, du Bois-Reymond Marcus 1944, Kaczmarek et al. 2015). However, we only found a single specimen and a detailed observation of the morphological traits was not possible and *A. scoticum* is considered a complex of species and a reanalysis of the taxon is necessary with an integrative taxonomy approach (Kaczmarek et al. 2015, Duenäs-Cedillo et al. 2020). Thus, we decided to keep as *Adropion* sp.

The last morphotype, *Echiniscus* sp. is very similar to *Echiniscus succineus* Gasiorek & Vončina 2019, only reported for the type locality in Madagascar. However, the specimen found in Serra do Japi differs from *E. succineus* by the presence of a small denticle on spines D<sup>d</sup> (absent in *E. succineus*) and lacks epicuticular thickenings at the edges of the dorsal plates (present in *E. succineus*). Given these morphological differences and considerable geographic distance, they are probably different species.

It is known that Brazilian freshwater and limnoterrestrial meiofauna organisms are poorly studied and their biodiversity is still underestimated (Garraffoni et al. 2017a, Kaczmarek et al. 2015, de Barros RC 2020). This perspective will only change if the number of surveys increases in the coming years. As the number of taxonomists specialized in these groups is very low in Brazil, the opportunity to show these lesser-known taxa to undergraduate students can potentially inspire

the next generation of meiofaunal researchers. Despite some problems in sorting and identification routines due to students' inexperience, this study achieved important goals of teaching and provided an immersive experience for the students interested in these tiny animals.

## Acknowledgements

We are thankful to FAPESP (Fundação de Amparo à Pesquisa do Estado São Paulo - 2014/23856-0; 2018/10313-0; 2018/11166-0; 2018/25213-0) for financial support and ICMBio for the research permits (SISBIO n° 69660). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. We also acknowledge Yasmina Shah Esmacili for the English revision of the manuscript and Wagner Luiz dos Santos, Thamara Rodrigues dos Reis and Aurélio Fajar Tonetto for macrophytes and bryophytes identifications.

## Author Contributions

Emiliana Brotto Guidetti: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision.

Ariane Campos: Contribution in the concept and design of the study, data collection, data analysis and interpretation, manuscript preparation, and critical revision.

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André Rinaldo Senna Garraffoni: Contribution in the concept and design of the study, data collection, data analysis and interpretation, manuscript preparation, critical revision, and funding acquisition.

## Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

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Received: 24/11/2020

Revised: 06/02/2021

Accepted: 09/03/2021

Published online: 03/05/2021