Biodiversity Journal, 2023,14 (4): 673–687 https://doi.org/10.31396/Biodiv.Jour.2023.14.4.673.687

Further records and dating of *Pseudunio auricularius* (Spengler, 1793) (Bivalvia Margaritiferidae), from Cagnola Canal (Veneto, Italy)

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ABSTRACT *Pseudunio auricularius* (Spengler, 1793) (Bivalvia Margaritiferidae) is one of the largest European freshwater bivalves; it is considered critically endangered worldwide, and it is extinct in Italy. A large number of damaged ancient shells have been collected in 1991 from Cagnola Canal (Province of Padua, Veneto, Italy) and radiocarbon dated. The results indicate that the extinction of the local *P. auricularius* population in Veneto occurred in the second half of 1800. The direct anthropic impact may have been the source of significant disturbance to the local extinction of *P. auricularius*.

KEY WORDS Italy; mollusc extinction; *Pseudunio auricularius*; radiocarbon dating; Veneto.

Received 15.09.2023; accepted 20.11.2023; published online 30.12.2023

INTRODUCTION

Pseudunio auricularius (Spengler, 1793) (Bivalvia Margaritiferidae) (genus taxonomy according to Lopes-Lima et al., 2018; Zotin, 2018) is one of the largest European freshwater bivalves and it is considered critically endangered according to IUCN European Red List of non-marine molluscs (Araujo & Ramos, 2000c; Cuttelod et al., 2011). Living populations are currently known only in few basins of western France, including Loire, Charente, Dordogne and Adour, and in the Spanish Ebro basin (Altaba, 1990; Araujo & Ramos, 1998, 2000c; Cochet, 2001; Nienhuis, 2003; Lopes-Lima et al., 2017; Prié, 2017). In northern Italy, the species became extinct between the end of 1800 and the beginning of 1900 (Manganelli et al., 2000; Biddittu & Girod, 2005; Stoch & Bodon, 2014).

The presence of recent specimens of *P. auricularius* was for the first time recognized in northern Italy in 1855 (De Betta & Martinati, 1855), where one specimen was identified. The historical reports concern, in northern Italy, the provinces of Mantua and Padua; in the latter province the sites are the Brancaglia Canal near Este and, most importantly, the Cagnola Canal (De Betta & Martinati, 1855; De Betta, 1870; Biddittu & Girod, 2005), where, at the beginning of the mid–19th century, several specimens were collected, now preserved in the collection of several European museums (Castagnolo & Nagel, 1994; Araujo & Ramos, 2000b; Castagnolo et al., 2002).

In 1991 in the Cagnola Canal, after the collection of sediments by dredging machine, several old broken valves of *P. auricularius* were found on the bank (Niero & Bodon, 2011).

The simultaneous decline of the common sturgeon *Acipenser sturio* Linnaeus, has been suggested among the causes of the decline of *P. auricularius* in Europe. Fossil remains of this bivalve were recovered together with those of *A. sturio* and it has been ascertained that *Acipenser* infested by the larvae of *P. auricuarius* ensures them a good viability. The glochidia, as in all the Margaritiferids and the Unionids, require a period of parasitic life, attaching to the skin or the gills of the fishes, to complete their development (Araujo & Ramos, 1998, 2000a, 2000c; López & Altaba, 2006). Currently, the common sturgeon *A. sturio*, and the Ladan sturgeon *Huso huso* (Linnaeus) are practically extinct in Italian waters. In Padua, the last report of living sturgeon, *A. naccarii* Bonaparte, dates to 1967. This species has recently been subjected to repopulation in the waters of the Brenta and Bacchiglione rivers (Marconato et al., 1988; Zerunian, 2002; Turin, 2004; Fortini, 2016; Busatto et al., 2017). The extinction of the sturgeons may not be the only cause of the disappearance of *P. auricularius* because other fishes, such as the Blenniidae *Salaria fluviatilis* (Asso) or the Petromizontidae *Petromyzon marinus* (Linnaeus), can successfully host its glochidia (López & Altaba, 2006; Soler et al., 2019). However, the presence of *S. fluviatilis* has never been observed in the waters of the basin of the Bacchiglione River, whereas *P. marinus* is also almost extinct (Turin et al., 1995; BIOPROGRAMM et al., 2021).

MATERIAL AND METHODS

Old shells of *P. auricularius* were collected by hand directly from the bank of the canal, where sediment dredged by mechanical means from the bottom of the channel was deposited. Further research in the sediments on the banks or in the bottom of the Cagnola Canal or other canals of the Padua area has not led to other findings whereas, in these channels, living specimens of other Unionidae as *Microcondylaea bonellii* (Férussac), *Anodonta* cf. *exulcerata* Porro, and *Unio elongatulus* Pfeiffer (species taxonomy according to Marrone et al., 2019) have been collected. The material illustrated in the present work is preserved in the following collections: Natural History Museum of the University of Florence, “La Specola” Zoology section, Florence, Italy (MZUF); Marco Bodon, Genoa, Italy (MBC). Other specimens are stored in the Museum d’Histoire Naturelle, Geneva, Switzerland (MHNG), and in the Museo di Storia Naturale di Verona, Italy (MSNVR).

Radiocarbon dating (C^{14}) was conducted on two fragments of *P. auricularius*. Because these specimens exchange carbon with the water of the canal during their life cycle, and do not directly interact with the atmosphere, they may be subjected to the so-called ‘hard water effect’ (Ervynck et al., 2018). Therefore, living specimens of other bivalves were also dated (two valves of *Unio elongatulus* and one valve of *Microcondylaea bonellii*) to calculate the necessary correction for the measured radiocarbon concentrations of *P. auricularius*.

Collecting sites of* Pseudunio auricularius *(Spengler, 1793) and other Unionidae (Fig. 1)

ITALY • Region Veneto, Prov. Padua, Municipality Cartura, Cagnola Canal, 600 m upstream Cagnola; UTM ED50 32T 0724600 5019100; 5 m a.s.l.; 21 Jun. 2001; M. Bodon leg. (shells of *M. bonellii* and *U. elongatulus*).

ITALY • Region Veneto, Prov. Padua, Municipality Cartura, Cagnola Canal, at Cagnola; UTM ED50 32T 0725250 5018900; 5 m a.s.l.; 13 Sep. 1990; M. Bodon leg. (shells and living specimens of *M. bonellii* and *U. elongatulus*); M. Bodon leg. 15/09/1991 (old shells of *P. auricularius* and shells of *M. bonellii* and *U. elongatulus*).

ITALY • Region Veneto, Prov. Padua, Municipality Cartura, Cagnola Canal, at Gorgo; UTM ED50 32T 0727900 5018050; 4 m a.s.l.; 13 Sep. 1990; M. Bodon leg. (shells and living specimens of *M. bonellii* and *U. elongatulus* and 1 shell of *Anodonta* cf. *exulcerata*.

ITALY • Region Veneto, Prov. Padua, Municipality Bovolenta, Cagnola Canal, upstream Bovolenta; UTM ED50 32T 0729350 5017620; 3 m a.s.l.; 25 sep. 87; M. Bodon leg. (shells and living specimens of *U. elongatulus* and 1 shell of *A.* cf. *exulcerata*); 13 Sep. 1990; M. Bodon leg. (shells of *M. bonellii* and *U. elongatulus*).

The Cagnola Canal is a large artificial channel that drains the area of the Euganean Hills where thermal springs also flow; it collects the waters of the Vi-

Figure 1. Map showing the sampling site of old shells of *Pseudunio auricularius* (Spengler) (red circle) and other sampling sites of fresh shells or living Unionidae (yellow circles) (see sampling sites for the data on localities).

genzone Canal, which in turn collects the waters of the Bisatto Canal and the Battaglia Canal, and other lateral channels to this. It then flows towards Bovolenta where it enters the Bacchiglione River (Turin et al., 1995). There is no precise information on the time of the excavation of the Cagnola Canal but, certainly, this watercourse was already present at the date of the excavation of the Bisatto and Battaglia canals, in the twelfth and thirteenth centuries, because their currents have opposite directions and therefore had to discharge into it. However, before this period, there could have been a drainage channel for sewage from the Euganean Hills, later rectified (Emanuele Martino *in litteris* 06/2022). From a chemical point of view, the waters of the Cagnola Canal currently appear now somehow compromised. However, until the end of the last century it still hosted a rich fish community today very degraded by the presence of many alien species (Turin et al., 1995; BIOPROGRAMM et al., 2021).

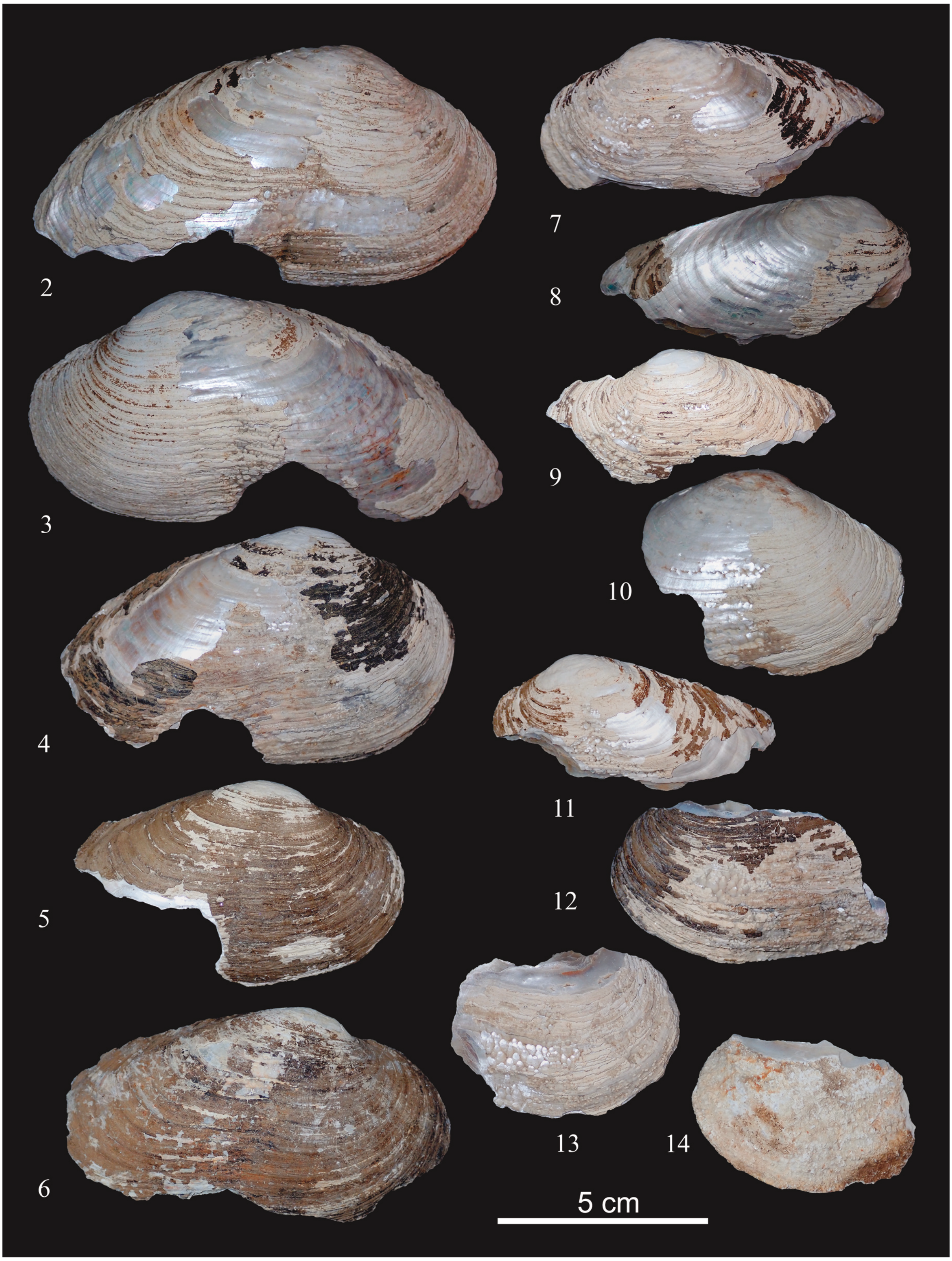
Collected material (Figs. 2–27)

Forty-four (44) valves of *P. auricularius*, 18 right and 26 left, were collected. All the valves are either broken or in small fragments, and several show periostracal residues. Two valves are almost complete with only one rupture in the basal-posterior margin (Figs. 2, 3, 15–16). Other eleven valves have wider ruptures along the margin and on the posterior part (Figs 4–5, 3 17–18). Four valves have breaks extending to the front (Figs. 6, 7, 19–20). Still twelve fragments are provided with umbo (Figs. 8–11, 21–24) and other 15 fragments without or almost without umbo (Figs. 12–14, 25–27). No valves or fragments appear to belong to the same specimen. The length of the two almost complete valves is 113 and 124 mm. Based on the other valves provided with lateral tooth, the smaller specimen is estimated to be about 80–85 mm long.

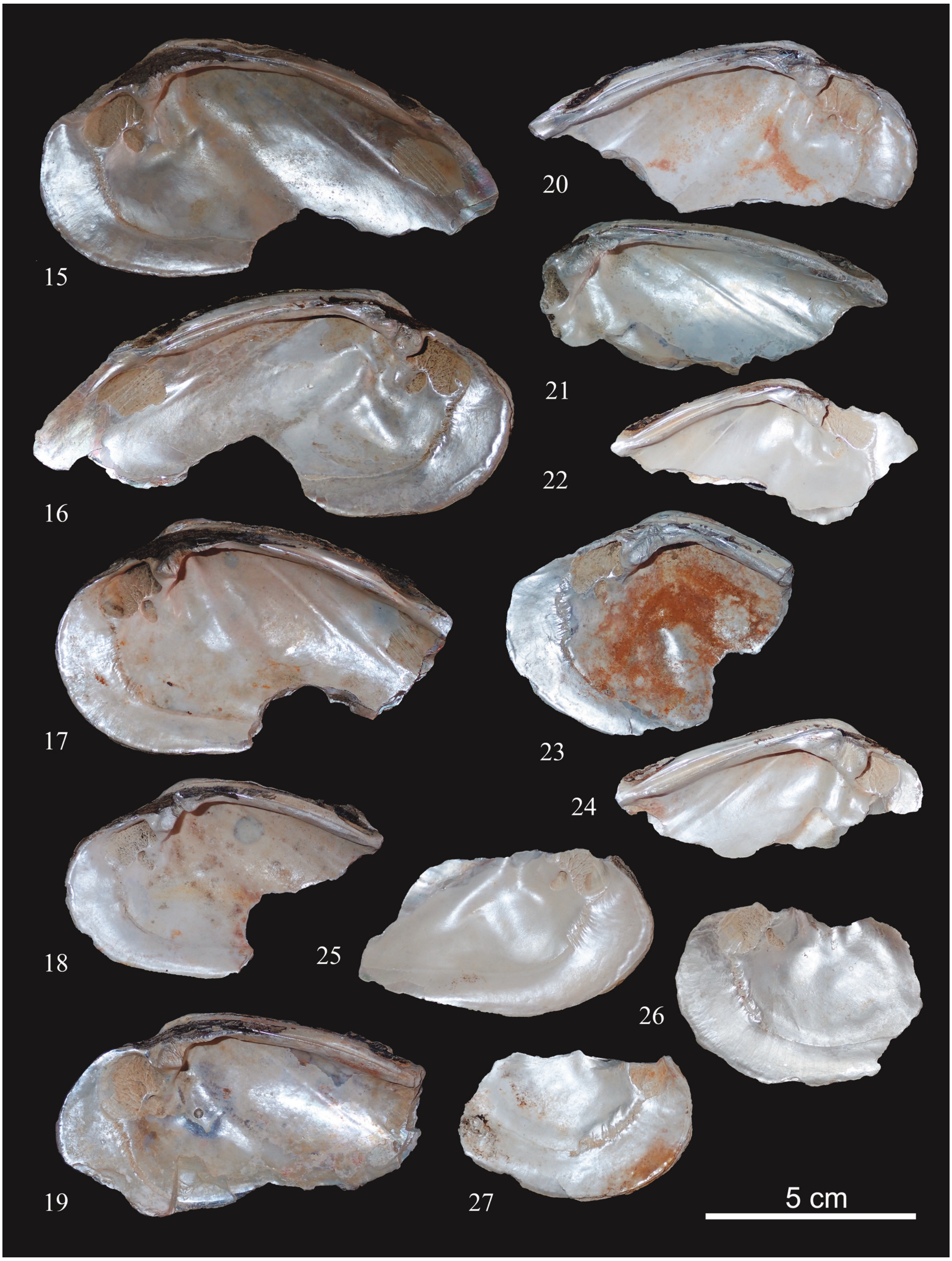
After comparing of the Cagnola Canal collected specimens in 1991 with the sizes of the historical ones (Table 1; Figs. 28–33), it appears that our more recent finds consist of adult or subadult specimens.

Collection or literature	Length (mm)
MHNG, Araujo & Ramos, 2000b	108
MZUF, ex Paulucci (specimen BC/1793, Figs 28–33)	133
MSNVR, ex De Betta	113–140
Tommasi (1875)	115–140

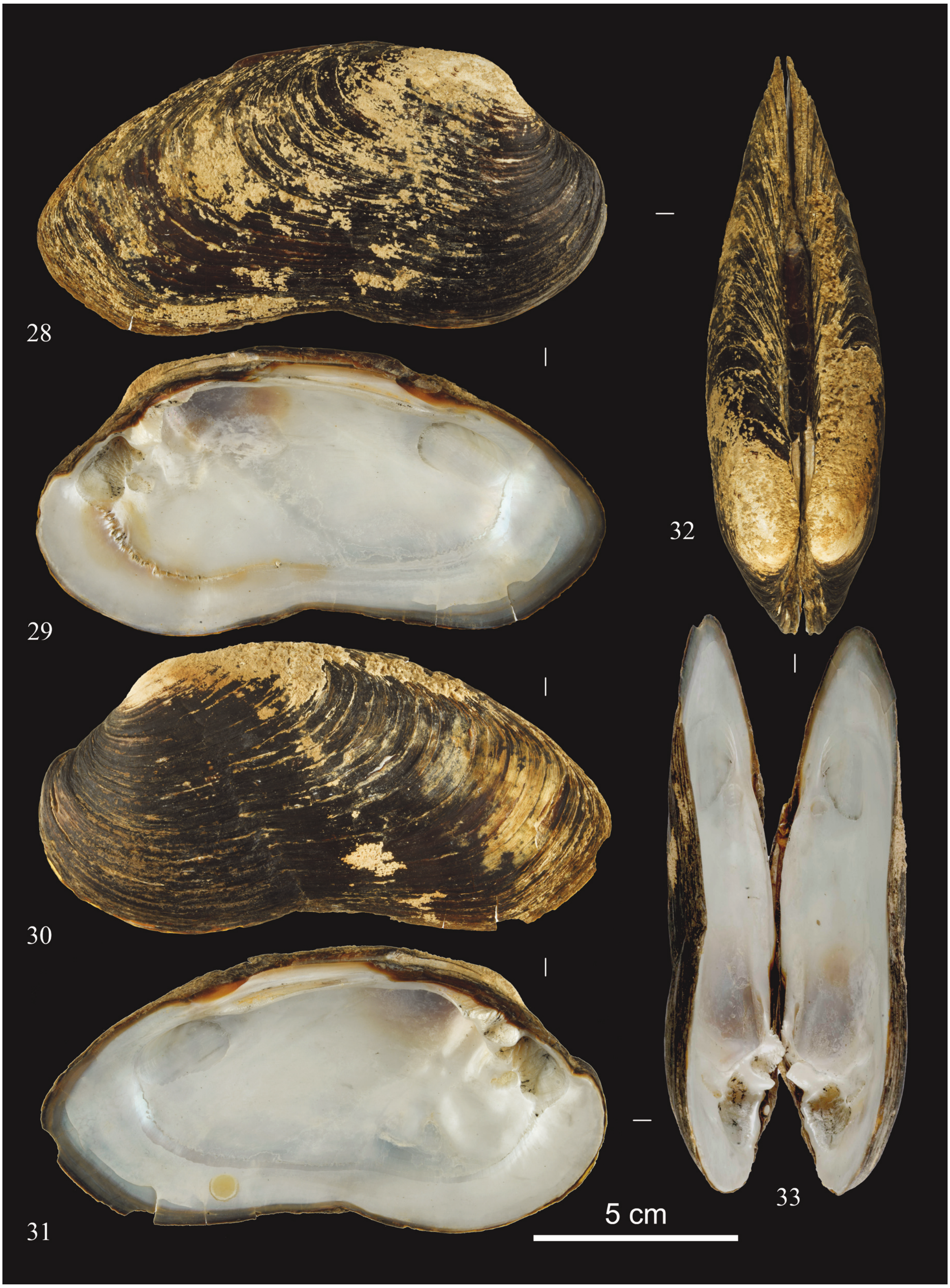
Table 1. Length of the specimens of *Pseudunio auricularius* from Cagnola Canal, available in a few historical collections and literature.



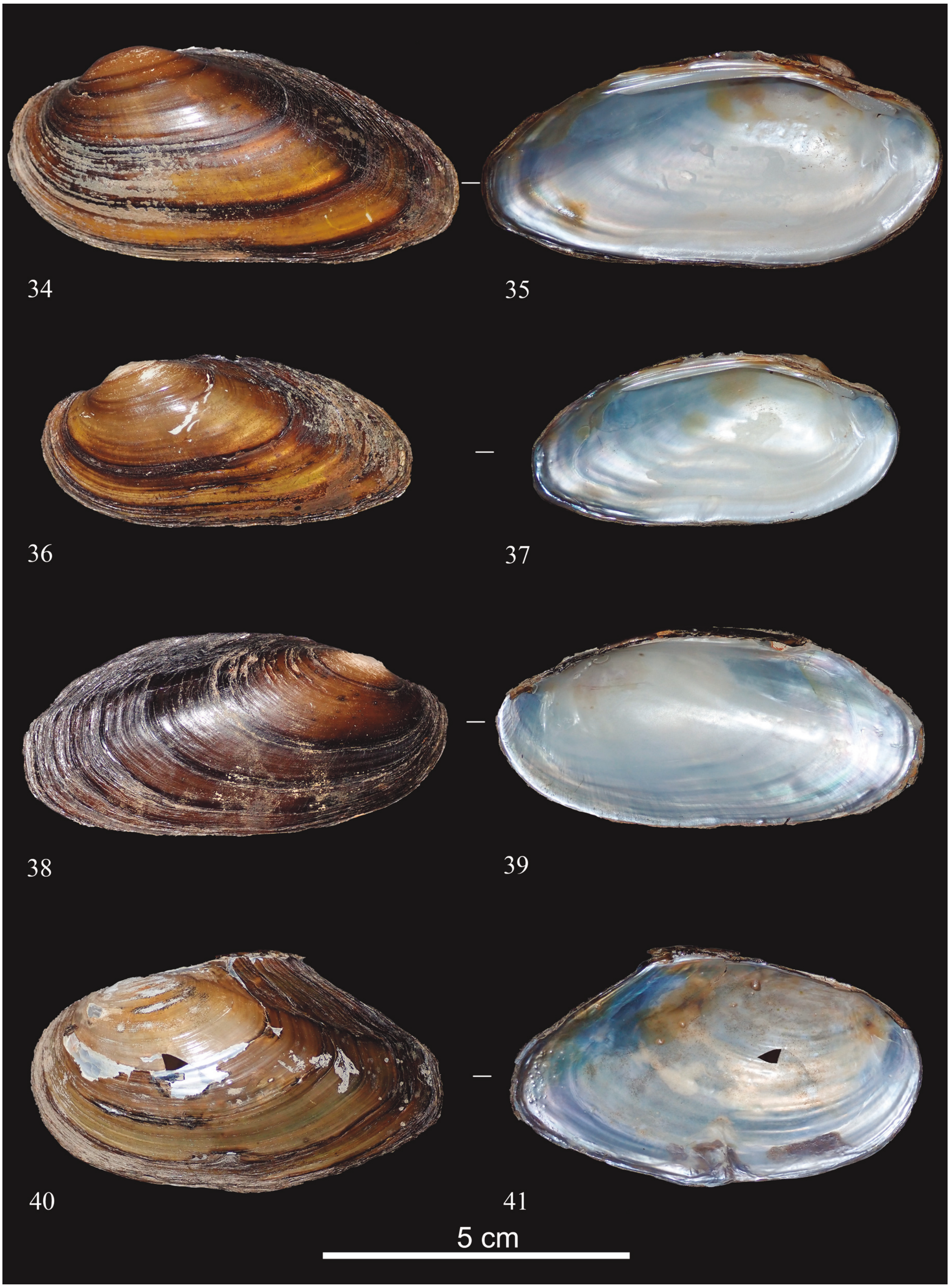
Figures 2–14. Old shells of *Pseudunio auricularius* (Spengler), in external view, from Cagnola Canal, at Cagnola site. Figs. 2, 3: fairly complete valves. Figs. 4, 5: valves that have only one rupture in the basal tract and along the posterior margin. Figs. 6, 7: valve with breaks extended also to the front. Figs. 8–11: fragments with umbo. Figs. 12–14: fragments without umbo. Figs. 2–10, 13, 14: MBC. Figs. 11, 12: samples processed for radiocarbon dating.



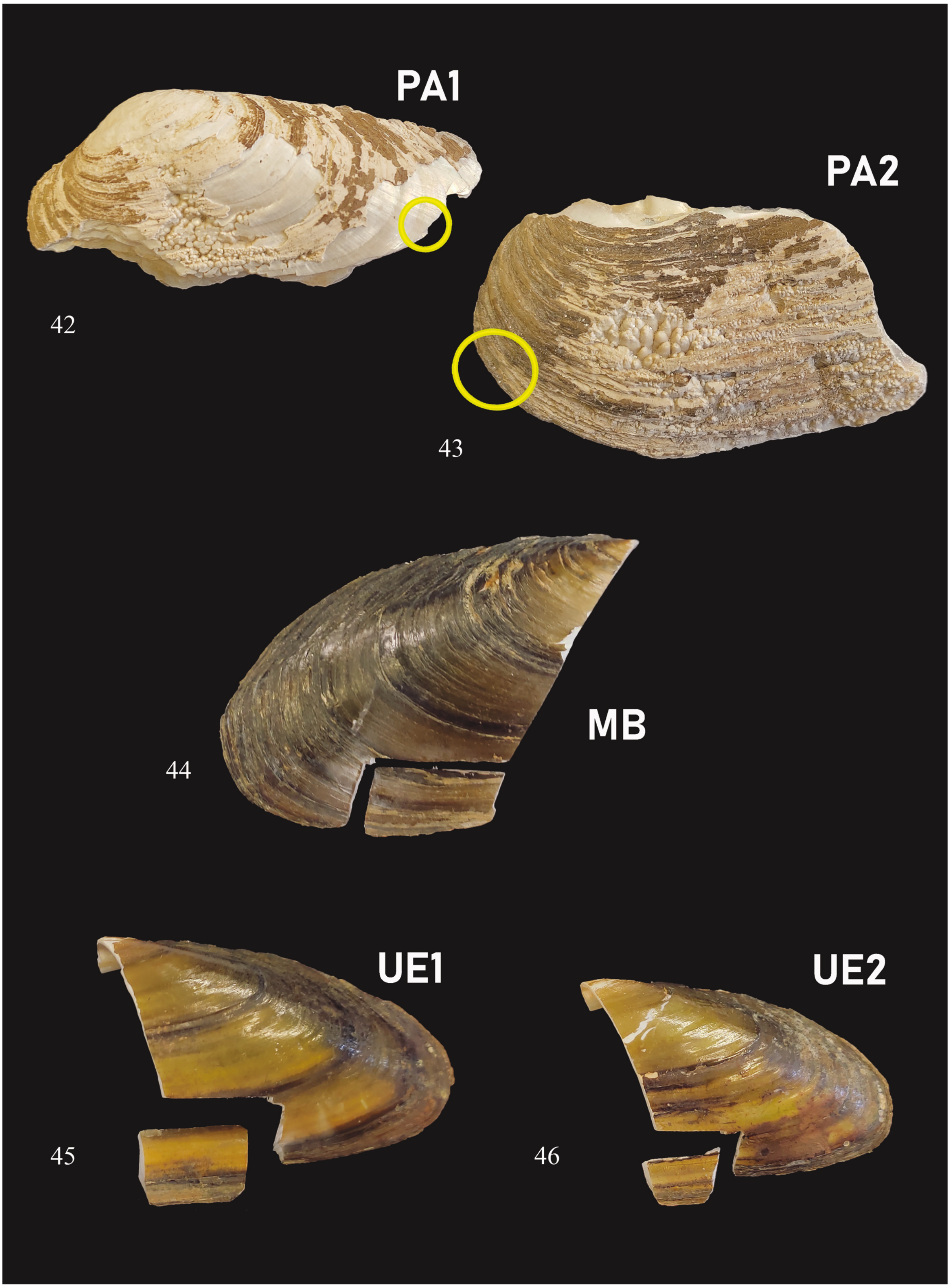
Figures 15–27. Old shells of *Pseudunio auricularius* (Spengler), in internal view, from Cagnola Canal, at Cagnola site. Figs. 15, 16: fairly complete valves. Figs. 17, 18: valves that have only one rupture in the basal tract and along the posterior margin. Figs. 19, 20: valve with breaks extended also to the front. Figs. 21–24: fragments with umbo. Figs. 25–27: fragments without umbo. Figs. 15–23, 26, 27: MBC. Figs. 24, 25: samples processed for radiocarbon dating (PA1 and PA2).



Figures 28–33. Shell of living *Pseudunio auricularius* (Spengler) from Cagnola Canal, near Gorgo (Cartura, Padua), De Betta leg., 1861, Paulucci collection, MZUF BC/1793.



Figures 34–41. Recent shells of other Unionidae from Cagnola Canal, M. Bodon leg. 13/09/1990. Figs. 34, 35: *Unio elongatulus* Pfeiffer, 1825, from Gorgo. Figs. 36, 37: *U. elongatulus* from Cagnola. Figs. 38, 39: *Microcondylaea bonellii* (Férussac, 1827) from Cagnola. Figs. 40, 41: *Anodonta* cf. *exulcerata* Porro, 1838, from Gorgo.



Figures 42–46. Shell fractions sampled for the radiocarbon measurements. PA1: *Pseudunio auricularius*, sample 1; PA2: *Pseudunio auricularius*, sample 2; MB: *Microcondylaea bonellii*; UE1: *Unio elongatulus*, sample 1; UE2: *Unio elongatulus*, sample 2.

Radiocarbon dating - Analysed samples

Table 2 summarizes the processed samples. MB, UE1 and UE2 (Figs. 34–45) were chosen to estimate the possible hard water effect. They were collected in two different sites along the Cagnola Canal, but the distance between the two sites is only 2.9 km. Also considering the limited mobility of adult individuals of such bivalves, we can neglect possible differences due to the different sites. When collected in September 1991, MB, UE1 and UE2 were alive, so we can assume that the carbon in the most external layer reflects the radiocarbon concentration in atmosphere of that year. To estimate the hard water effect, this part is the most appropriate shell fraction to be collected and measured. However, in our samples, these external layers were very degraded and corresponded to a very low mass, well below the typical amount of carbonate mass processed for radiocarbon measurements (of the order of 10 mg). Thus, from each of the shells, we decided to collect a portion including more than one year of growing, tentatively corresponding to about 1988–1990 (see Figs. 42–46 for the sampled fractions). The expected radiocarbon concentration in such collected samples is therefore an average of the radiocarbon concentrations in atmosphere in those years.

Most external fractions from samples PA1 and PA2 were collected as well. For both individuals, however, the shell surface presented lot of carbonate concretions that might have introduced modern contaminations. Portions to be measured were thus sampled from the clearer surfaces.

Sample preparation and Accelerator Mass Spectrometry measurements

The first step in sample preparation aims at removing possible contaminations. In the present case, the most external layers of calcium carbonates, which may be subjected to exchanges with the external environment and possible macro residues of organic matter were to be removed. Slightly different approaches were chosen for recent and old samples, since shells to be measured showed different levels of degradation. In particular, in addition to the already cited carbonate concretions, unknown samples PA1 and PA2 were very fragile and poorly preserved.

The whole collected pieces of samples MB, UE1 and UE2 underwent this cleaning procedure:

0.1 HCl at room temperature for 10 minutes;
0.1 NaOH at room temperature for 10 minutes;
0.1 HCl at room temperature for 10 minutes;
H_2O_2 at room temperature for 15 minutes.

On the contrary, samples PA1 and PA2 were only treated in H_2O_2 at room temperature for 15 minutes, to preserve them as much as possible.

Afterwards, all the samples were powdered in a mortar and eventually CO_2 was extracted from each of them by dissolution in orthophosphoric acid (H_3PO_4). The dissolution tube is connected to the rest of the graphitization line where carbon dioxide is first purified from any water residues and finally converted to graphite by reaction of CO_2 with hydrogen, at high temperature and in the presence of iron as catalyst. Two graphite samples were prepared from each of the treated shells (see Table 2 for the details about laboratory codes).

Species	**Sample**	**Lab. code**	**Cagnola collecting site**	**Collecting year**
Pseudunio auricularius 1	PA1	Fi5173, Fi5174	2	1991
Pseudunio auricularius 2	PA2	Fi5171, Fi5172	2	1991
Microcondylaea bonellii	MB	Fi5162, Fi5163	2	1990
Unio elongatulus 1	UE1	Fi5165, Fi5166	3	1990
Unio elongatulus 2	UE2	Fi5167, Fi5168	2	1990

Table 2. Samples measured by radiocarbon: species, samples names and laboratory codes, collecting sites and years are indicated.

Radiocarbon concentrations were measured by Accelerator Mass Spectrometry (AMS) at the dedicated beam line of the Tandem accelerator of LABEC, Florence (INFN-CHNet) (Fedi et al., 2007). Samples prepared from SRM4990C, IAEA C2 and IAEA C1 were used as primary standards, secondary standards and blanks, respectively. Correction for isotopic fractionation was also applied by measuring the $^{13}C/^{12}C$ ratio along the beam line as well.

Calibration of the measured radiocarbon concentrations was performed using OxCal software (Bronk Ramsey, 2009). Both IntCal20 (Reimer et al., 2020) and Bomb21NH1 (Hua et al., 2022) were considered for calibration and for evaluation of the possible hard water effect.

RESULTS

Table 3 shows the measured radiocarbon concentrations in both recent and old samples. For each of them, the best estimate of the concentration has been evaluated as the weighted average of the two measured graphite fractions (after checking that both fractions were consistent according to statistical analysis).

As a first comment, one about the possible hard water effect can be done. In 1991, the radiocarbon concentration in atmosphere was about 114–116 pMC, according to the well-known Bomb peak.

This refers to the peculiar behaviour of C^{14} concentration in atmosphere after 1950, characterized by a large and very rapid increase because of the nuclear tests and a following decrease after the Nuclear Ban Treaty. If the samples were not affected by hard water effect, we would have expected a concentration comparable to 114–116 pMC. However, the measured radiocarbon concentrations were below 100 pMC, suggesting an apparent ageing due to the different carbon reservoir the bivalves exchanged with. The comparison of such measured concentrations with the Bomb peak calibration curve Bomb21NH1 allow us to estimate the hard water correction to be eventually summed to the measured C^{14} concentrations of samples PA1 and PA2 to obtain the corrected values. To estimate the correction ΔR, these criteria were followed:

each of the samples is constituted by more than one year of growth, all of them being prior to 1991; since, as already discussed, the most external layer was very poor, we decided to neglect its contribution to the overall reference radiocarbon concentration in atmosphere (^{14}C conc. ref. atm.);

for each of the samples, ^{14}C concentration reference in atmosphere is calculated as the weighted average of the concentrations in atmosphere in the months associated to the collected growth layers. The weights were chosen according to the thickness of the layers themselves.

Estimated corrections are reported in Table 4. A slight scattering between the ΔR, not fully compatible within the experimental errors, can be noticed. Nevertheless, we cannot exclude that a certain variability can be associated to possible differences in metabolism of each of the individuals. Thus, to have a rough estimation of the correction, we decided to evaluate ΔR_{avg} as the average of the estimated corrections shown in Table 4: $\Delta R_{avg} = 21.1 \pm 0.8$.

Accordingly, ΔR_{avg} was used to correct the

Sample	^{14}C conc. (pMC)
MB	94.91±0.49
UE1	98.95±0.45
UE2	95.88±0.40
PA1	78.48±0.40
PA2	81.69±0.41

Table 3. Measured radiocarbon concentrations, expressed as percentage (%) of Modern Carbon.

Sample	^{14}C conc. ref. atm. (pMC)	ΔR
MB	117.65 ± 0.83	22.7±1.0
UE1	118.36 ± 0.83	19.4±0.9
UE2	117.15 ± 0.32	21.3±0.5

Table 4. ΔR corrections as determined by comparison of the measured radiocarbon concentrations of samples MB, UE1 and UE2 and the expected reference radiocarbon concentration in atmosphere from the tabulated Bomb21NH1.

measured radiocarbon concentrations for old samples PA1 and PA2, as reported in Table 5. In addition to the corrected concentrations, Table 5 also shows the calibrated time intervals, at 68% and 95% level of probability, respectively. Considering that the radiocarbon concentrations are about 100 pMC, a value marking a sort of separation between the use of IntCal20 and Bomb21NH1, calibrated time intervals are reported as the union of the periods estimated by comparison with both the mentioned calibration curves (Figs. 47 and 48–49). Considering that the distributions of probability for the calibrated ages (Figs. 47 and 48–49) cannot be analytically described, time intervals determined according to fixed levels of probability (or confidence) 68% and 95% are typically indicated as results of the dating measurements (Table 5).

DISCUSSION

In Italian waters, the link between *Acipenser sturio* and *P. auricularius* is almost certain, whereas this is more uncertain with other fish species. The disappearance caused by overfishing of the former species inevitably has repercussions on the latter, which in the case of *P. auricularius* glochids no longer have any viability. Other causes, again of an anthropic nature, concern the alteration of riverbeds, the removal of river sediments, and the construction of dams hindering the migration of fish could have played a significant role (Manganelli et al., 2000). The impacts of anthropogenic origin, which may have played a relevant role in the decline of *P. auricularius,* could also be water pollution, the indiscriminate collection of shells to extract river pearls, to obtain mother-of-pearl or for collection purposes. Furthermore, since prehistoric times, the valves of this mollusc have been used to obtain artifacts (Biddittu & Girod, 2005; Girod, 2010; Borrello & Girod, 2010.).

All specimens of *P. auricularius* collected from Canale Cagnola are broken but the ruptures of the valves do not reflect those shown on *Unio tumidus* Philipsson, 1788 (Collet, 2015) produced by predation of birds or rats, but are like those observed on broken specimens of *Margaritifera margaritifera* (Linnaeus, 1758) to extract for pearls, as in Scotland (Willing, 2020).

Focus must be drawn to the case of sample PA2 (Figs. 48–49). The measured radiocarbon concentration above 100 pMC might suggest a calibration to a period after 1955. On Fig. 48, after 1955, two-time intervals are identified. In particular, the most recent one can be neglected since it is subsequent the date of collection in 1991. The tail of the distribution of the measured radiocarbon age is however below 100 pMC and thus a short period identified in the period before 1955 is found as well (see Fig. 49).

Results may suggest that *P. auricularius* individuals were still present in the first half of the 20th century. The ^{14}C dating of the retrieved od valves from the Cagnola Canal confirms that the past local intentional anthropic impact, acting with an indiscriminate herd of living individuals of different size, led to the disappearance of *P. auricularius* in Italy.

CONCLUSIONS

The dating results allow us to state with some reliability that the finds of *P. auricularius* are contemporary or slightly later than the historical reports from the second half of the 19th century. It is hypothesised that there was an important anthropic impact on this population, perhaps for pearl hunting, although there is no written evidence. Food use

Pseudunio auricularius	**^{14}C conc. corr. (pMC)**	**Calibrated age (68% prob.)**	**Calibrated age (95% prob.)**
PA1	99.6±0.9	1690–1730 1810–1920 1950–1955	1670–1780 1795–1955
PA2	102.8±0.9	1925–1960	1890–1960

Table 5. Radiocarbon concentrations of *Pseudunio auricularius* samples PA1 and PA2, as corrected for the hard water effect, and corresponding calibrated time intervals.

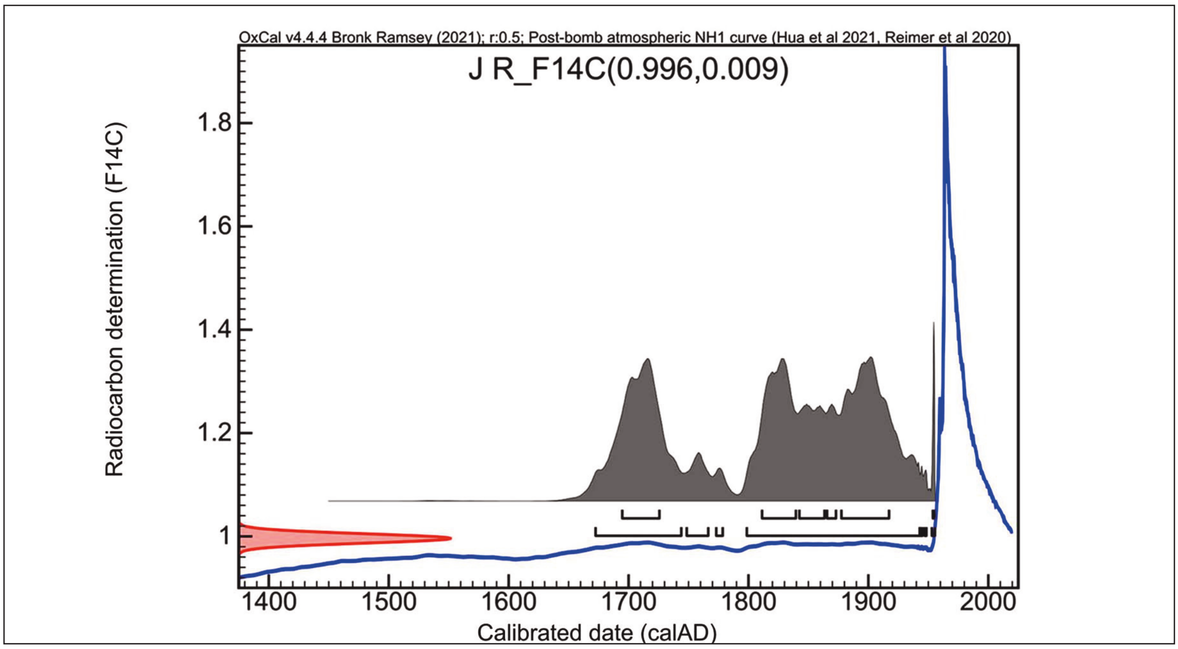


Figure 47. Calibration graph of the measured radiocarbon concentration of sample PA1 (calibration obtained by measured radiocarbon concentration with Bomb21NH1 + IntCal20). The calibration curves, either IntCal20 or Bomb21NH1 or the union of both, are reported in blue; the corrected radiocarbon concentration are represented as normally distributed random variables and shown in red on the y axis; the calculated distributions of probability for the calibrated ages are reported in grey on the x axis.

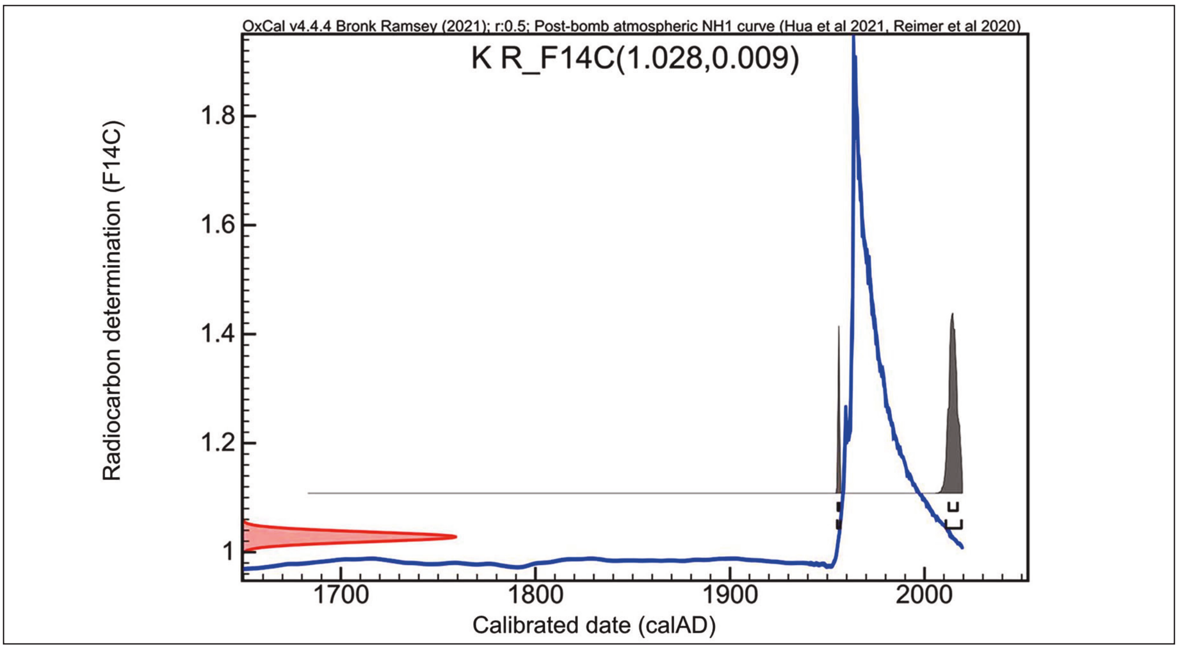


Figure 48. Calibration graph of the measured radiocarbon concentration of sample PA2: calibration obtained by comparison of the measured radiocarbon concentration with Bomb21NH1. The calibration curves, either IntCal20 or Bomb21NH1 or the union of both, are reported in blue; the corrected radiocarbon concentrations are represented as normally distributed random variables and shown in red on the y axis; the calculated distributions of probability for the calibrated ages are reported in grey on the x axis.

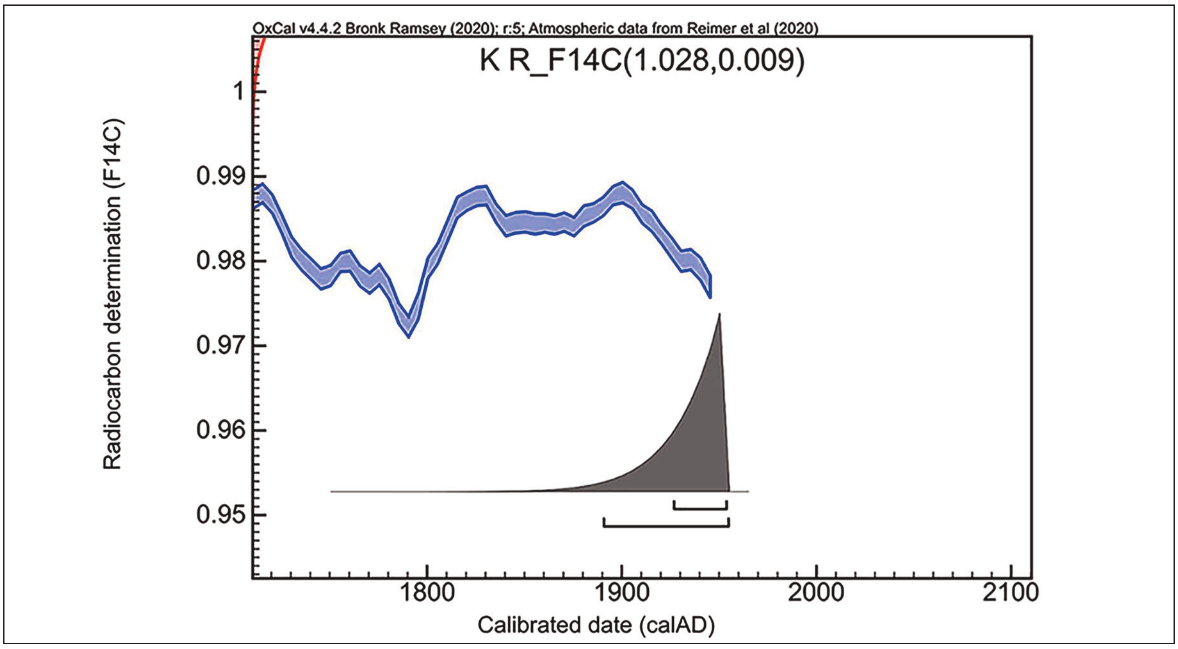


Figure 49. Calibration graph of the measured radiocarbon concentration of sample PA2: calibration obtained by comparison of the measured radiocarbon concentration with IntCal20. The calibration curves, either IntCal20 or Bomb21NH1 or the union of both, are reported in blue; the corrected radiocarbon concentrations are represented as normally distributed random variables and shown in red on the y axis; the calculated distributions of probability for the calibrated ages are reported in grey on the x axis.

is not ruled out, based on what is known about the use of freshwater bivalves in the cuisine of northern and central Italy (Girod, 2015).

ACKNOWLEDGEMENTS

We thank Emanuele Martino (Ponte San Nicolò, Italy) for the historic information on Cagnola Canal; Paolo Turin (Padua, Italy) for the information on the fish fauna and to PhD Daeei Sassoon for revising the English text. The research was funded with contributions from LABEC Laboratory of Nuclear Techniques for the Environment and Cultural Heritage, INFN Section of Florence Department of Physics and Astronomy, University of Florence. and financed by the Natural History Museum, Zoological Section "La Specola", University of Florence (Italy).

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