Report of the project "Rescuing *Microcondylaea bonellii*: a testimonial for non-iconic neglected species" - Report September 2021

Nicoletta Riccardi

CNR – Water Research Institute, Verbania (Italy)

Due to the COVID-19 health emergency, the project's action plan could not unfold as planned. However, even with all the barriers associated with this pandemic, the following activities have been performed**:**

Activity 1

Assessment of distribution, status and genetic variability of extant populations over whole range.

Status

A new assessment with the purpose to change the conservation status of *Microcondylaea bonellii* from Vulnerable (VU - A2c) to Critically endangered (CR - A2ac), was submitted to IUCN (Riccardi and Lopes-Lima, submitted) and it is under revision.

Assessment of distribution

In the station that tested positive for the environmental DNA analysis performed by Vincet Prié last year, an *in situ* search was carried out. The positive data confirms that the species is still present in a stretch of river that is upstream of the sampling. According to the literature relating to the persistence of DNA in the environment, the maximum distance from the water sampling point at which animals are most likely to be found is 1 km. Therefore, the survey was organized in the river about 1 km long upstream from the sampling point (Fig. 1 A). Although we had waited for the summer to have more favourable conditions, the high rainfall this year did not allow the usual decrease of the

water level and consequent decrease of the current speed. The Ticino Park provided logistical support by providing the team of volunteer ecological guards and the boat for the exploration of the identified river stretch (Fig. 1 B). The search was carried out both from the shore along the banks and in the more central areas of the river. Exploration from the shore was not effective because the high water level did not allow to reach the sediment. The exploration in the deepest and most central parts of the river was carried out by scuba diving (Fig. 1 C). In addition to me, two divers who volunteered to support me in this research dived. Since the current was very strong, to be able to explore the riverbed we had dived with an excess of ballast and holding on to a 50-meter rope tied to the support boat with one hand. Five dives were made 200 meters apart to cover 1 km upstream of the e-DNA analysis site. In order not to overlook any type of habitat, an oxbow lake still partially connected to the river was also explored. Despite the high sampling effort, no specimens of *Microcondylaea bonellii* were found. Unfortunately, this species is very difficult to locate because it is generally completely sunk in the sediment especially in the presence of a strong current. However, it is not excluded that the failure to find is due to the difficult sampling conditions, so we will repeat the attempt in the next dry season.

The search has raised the interest of the local press (Fig. 3).

A

B

C

D

Fig. 1 – A) The stretch of river explored; B) the support boat; C) beginning of a dive; D) final dive near the e-DNA discovery point.

Since the species was historically also reported in the network of irrigation canals of the Certosa di Pavia (De Betta, 1884), a monastery and sanctuary from the 1400s, we made an inspection courtesy of the monks (Fig. 2). No specimens have been found in the canals, but we have not been able to verify their possible presence in the fish pond inside the walls of the monastery. The fish pond is an artificial pond fed by the water of the canals, with a length of about 80 meters, a width of 20 and a depth of about 5 meters. The high turbidity of the fish pond prevented the visual inspection of the seabed by snorkeling. A search will be carried out by scuba diving during the cold season, when the water is clearer, to have better visibility.

A

B

C

D

E

Fig. 2 – A) A canal inside the monastery; B) The monumental complex of the Certosa (the red arrow indicates the fishpond); C) D) E) the fish pond.

Activity 2

Collection and culture of individuals under controlled conditions to optimize the percentage of fertilization and/or collection of pregnant females from donor populations.

Microcondylaea. Bonellii individuals, which were kept in the artificial river of IRSA-CNR (Istituto di Ricerca sulle Acque), were transferred into aquariums with flow through water pumped from Maggiore Lake and artificial sediment. As the eggs should mature starting from the second half of April, from the beginning of March we have increased the daily food ration and gradually increased the temperature in the aquariums. In addition, to diets already tested, as spirulina in powder, natural detritus from terrestrial vascular plants and natural mixture of algae from the Maggiore lake, *Nannochloropsis* sp. algae were also introduced in the diet of *M. bonellii* individuals to improve the nutritional conditions. The aim was to induce the production of glochids to initiate artificial reproduction bringing the animals in the onset of their pregnancy to a specialist in captive breeding, Dr. Karel Douda (University of Prague, Czech Republic).

Unfortunately, at the beginning of April I had to undergo surgery for an accident and at the same time my two collaborators were infected with COVID. Hence, activities slowed down. Furthermore, it was not possible to organize an air transport of the animals from Italy to Prague in a sufficiently short time to guarantee the survival of the animals. Fortunately, when the Italian government reopened the borders without quarantine (May 16), a colleague from the University of Poznan, Poland (Maria Urbanska) reached my laboratory in her car to bring the animals to Prague.

Activity 3

Culture of glochidia in: A) artificial media (test a wide range of media types); B) on putative fish hosts.

In spite of the many difficulties, the animals were delivered to the University of Prague on 24 May 2021. Although the animals were in good general condition upon arrival, none were pregnant. It is impossible to say if there were difficulties in mimicking the pre-reproductive conditions or if the maintenance in captivity does not allow the development of the glochids. After the arrival in Prague and the inspection for the verification of pregnancy, despite all the treatments, the animals suffered a mortality of 50%. Currently, only ten surviving animals are kept in the aquariums of the University of Prague.

Unfortunately, we know too little about this species to be able to recreate in captivity the conditions suitable for both survival and reproduction. After the experience of these two years of failed attempts, we have decided to adopt a different strategy. Since the chances of locating and collecting a sufficient number of animals in Italian sites are very low, we decided to organize a collection at two sites in the Balkan Peninsula: the Vipacco stream (Sloveni and the Mirna river (Croatia). Both sites should host populations abundant enough to allow the collection of a large enough number of individuals, and both sites have easy access points. Therefore, we have decided to organize an expedition in the next month of May requesting support from local researchers both to obtain permits and to identify the most suitable time with low water levels in rivers. The collected animals will be taken by car directly to the University of Prague to minimize the risk of mortality and early release of the glochids. To support this activity we will ask for an additional

financial contribution to the COST action CONFREMUS, e.g. applying to obtain a short term scientific mission.

References

De Betta E., 1884 - Sulle Najadi dell'Italia. Nota critica. Atti Ist. Ven. Scienze Lett. Arti, Venezia, Ser. VI, 2: 465-488.