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Title: Integrating RPA-LFD and TaqMan qPCR for Rapid On-Site Screening and Accurate Laboratory Identification of *Coilia brachygnathus* and *Coilia nasus* in the Yangtze River

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The work entitled "Integrating RPA-LFD and TaqMan qPCR for Rapid On-Site Screening and Accurate Laboratory Identification of *Coilia brachygnathus* and *Coilia nasus* in the Yangtze River" concerns development of the two complementary methods for fish identification i.e. RPA-LFD and TaqMan qPCR. RPA-LFD was used for *Coilia* species identification and the results of this method were received, with the application of the proper reagents, after 10 minutes samples incubation in 37 °C. In this method the primers and the probe designed on the base of COI were applied. Next the endpoint PCR and Sanger sequencing were made. On the base of the received sequences, SNP conservation and specificity were verified. The last step of the experiment was developing a dual TaqMan MGB qPCR. As a result, the developed method made possible to distinguish the *Coilia brachygnathus* and *Coilia nasus* in a one reaction tube. In a case of *Coilia brachygnathus* Ct values in the FAM channel appeared significantly earlier than those in the VIC channel with the detection limit of 125 copies per reaction. For *Coilia nasus* only the VIC channel amplification was observed with the detection limit 12.5 copies per reaction. The validation of the developed method confirmed 100 % consistency with the 171 known tissue samples.

The presented study is very interesting and certainly needed, as the authors indicated, for the fishery management, conservation and fight against food market adulteration. A particularly interesting solution was elaboration two complementary methods, one (RPA-LFD) which allows for the rapid on-site identification of fish belonging to the *Colia* species, and the other (TaqMan qPCR) permitting for the precise differentiation of *Colia nasus* from *Colia brachygnathus* in laboratory conditions.

This work fits the objectives and scope of the journal "Foods". The literature was selected properly and over 73 % quoted works came from the last 10 years. The research was carried

out properly and appropriate analyses were applied. Both the methodology and the research results have been described correctly but some minor errors appeared.

1. Page 4, line 18, section 2.3., the names of the primers are ”Cn-Cb-F”, “Cn-Cb-R” but in the page 5, table 2; page 5, section 2.6; page 6, section 3. Results, 3.1 etc. their names are different i.e ”Cb-Cn-F”, “Cb-Cn-R”. It looks like lack of the consequence in nomenclature.
2. Page 4, figure 1, the description of the figure is not clear. What mean the abbreviations: “C. nasus-Pe”, “C. nasus-Pn”, “C. nasus-Re”? Why “C. nasus-Pe” and “C. nasus-Re” are not presented in the table 2?
3. The tests presented in the paper were performed only on raw samples? It would be interesting to test the elaborated method on heat-treated samples too.
4. Page 2, line 5, in the sentence “However, populations of *C. nasus* have declined severely due to overfishing, habitat degradation, and river fragmentation, leading to its designation as Endangered (EN) on China's Biodiversity Red List [8, 9]” double space after word “However”.
5. Page 2, line 11 “While this supports *C. nasus* conservation, it has inadvertently facilitated commercial mislabeling, where farmed *C. brachygnathus* substitutes for high-value *C. nasus*, whether unintentionally or fraudulently [10]” double space after word “high-value”.
6. Page 2, line 13, “Consequently, accurate species discrimination is critical for sustaining stocks through evidence-based fisheries management, protecting consumers from economic deception, and ensuring market integrity” double space after word “sustaining”.

The above mistakes do not affect the positive opinion about the work. I will accept the submitted manuscript if the authors correct the minor errors indicated.