

Pengembangan Markah Gen NADH Dehydrogenase Subunit 5 (ND5) Spesies Spesifik untuk Deteksi Environmental DNA (eDNA) Kura-kura Rote Leher Ular (*Chelodina mccordi*, Rhodin 1994) dari Sampel Air = Development of Species-Specific NADH Dehydrogenase Subunit 5 (ND5) Gene Marker for Detection of Environmental DNA (eDNA) of Snake-necked Rote Turtles (*Chelodina mccordi*, Rhodin 1994) from Water Samples

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Abstrak

Kura-kura rote leher ular (*Chelodina mccordi*, Rhodin 1994) merupakan satwa endemik Indonesia yang hanya ada di Pulau Rote, Nusa Tenggara Timur. Tingginya perdagangan karena keunikan kura-kura rote berupa lehernya yang panjang disertai dengan hilangnya habitat menyebabkan status kura-kura rote menjadi critically endangered and possibly extinct in the wild. Upaya konservasi melalui program reintroduksi telah dilakukan, tetapi hasil pemantauan konvensional tidak menemukan kura-kura rote pada habitat aslinya. Pemantauan menggunakan environmental DNA (eDNA) dapat menjadi opsi alternatif karena deteksi dapat dilakukan berdasarkan spesifisitas dan sensitivitas primer. Penelitian bertujuan mengembangkan primer spesifik untuk deteksi *C. mccordi* dari sampel air dengan gen NADH Dehydrogenase Subunit 5 (ND5) sebagai target. Primer dirancang dengan ukuran pendek dan memiliki basa unik yang hanya ada pada *C. mccordi*. Primer diujikan pada air kolam dari *C. mccordi*, *C. expansa*, air campuran kedua spesies, serta air kontrol negatif yang tidak mengandung kedua spesies tersebut. Pengujian dilakukan melalui tahap filtrasi, ekstraksi, PCR, sequencing, dan qPCR. Hasil yang diperoleh menunjukkan primer ND5 (UI_Cm_ND5) berhasil mendeteksi *C. mccordi* dengan nilai sensitivitas 75% dan spesifisitas 100%. Hal tersebut menunjukkan primer ND5 dapat digunakan untuk mendeteksi eDNA *C. mccordi* dari sampel air. Penelitian lebih lanjut diperlukan untuk membandingkan sensitivitas dan spesifisitas primer ND5 dengan gen target lain.

.....The snake-necked rote turtle (*Chelodina mccordi*, Rhodin 1994) is an endemic animal to Indonesia that only distributed on Rote Island, East Nusa Tenggara. Uncontrolled trade due to the uniqueness of its long neck form accompanied by loss of habitat has caused the status of the rote turtles to become critically endangered and possibly extinct in the wild. Conservation efforts through a reintroduction program have been carried out, but traditional monitoring did not found their existence in natural habitat. Monitoring via environmental DNA (eDNA) can be an alternative approach as the detection based on the specificity and sensitivity of the species specific primer. This study aims to develop specific primers for the detection of *C. mccordi* from water samples with the NADH Dehydrogenase Subunit 5 (ND5) gene as the target. The primer was designed to be short and has a unique base that is only found in *C. mccordi*. The primer was tested on pond water from *C. mccordi*, *C. expansa*, water mixed with the two, as well as negative control water that did not contain these two species. Validation was performed through filtration, extraction, PCR, sequencing and qPCR steps. The results showed that the ND5 primer (UI_Cm_ND5) successfully detected *C. mccordi* with a sensitivity of 75% and a specificity of 100%. This result support the ND5 primer as genetic marker to detect the presence of *C. mccordi* eDNA from water samples. Further studies are needed

to compare the sensitivity and specificity of ND5 primers with other target genes.