Pengembangan Markah Gen Cytochrome B (Cyt b) Spesies Spesifik untuk Deteksi Enviromental DNA (eDNA) Kura-Kura Leher Ular Rote (Chelodina mccordi, Rhodin 1994) dari Sampel Air = Development of Species-Specific Cytochrome B (Cyt b) Gene Marker for Detection of Environmental DNA (eDNA) of Rote Snake-necked Turtles (Chelodina mccordi, Rhodin 1994) from Water Samples

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Abstrak

Kura-kura leher ular rote (Chelodina mccordi, Rhodin 1994) merupakan spesies endemik Pulau Rote, Nusa Tenggara Timur, Indonesia, dengan status konservasi kritis (critically endangered and Possibly Extinct in the Wild). Upaya reintroduksi kura-kura tersebut sudah dilakukan, tetapi keberadaan individu C. mccordi tetap tidak terdeteksi di alam. Pendekatan environmental DNA (eDNA) menjadi metode nonivasit alternatif untuk pendeteksian dan pemantauan spesies tersebut. Pengembangan metode pendeteksian eDNA C. mccordi memerlukan markah (primer) spesies spesifik agar dapat mengidentifikasi dengan akurat keberadaan DNA target pada sampel. Penelitian bertujuan untuk mengembangkan primer spesies spesifik untuk markah gen Cytochrome b (Cyt b) dalam pendeteksian eDNA C. mccordi. Primer dirancang berdasarkan urutan nukleotida gen Cyt b dari C. mccordi dengan spesies Chelodina lain. Pengujian primer dilakukan menggunakan sampel air yang mengandung DNA target untuk mengevaluasi spesifitas dan sensitivitas primer. Sampel air yang sudah terekstrak kemudian diproses dengan teknik Quantitative Polymerase Chain Reaction (qPCR) dan High Resolution Melting (HRM). Hasil penelitian menunjukkan bahwa primer desain (UI_Cm_Cytb) berhasil mengidentifikasi keberadaan C. mccordi dalam sampel. Nilai sensitivitas dan spesifitas primer tergolong tinggi, yaitu 87,5% dan 100%, serta primer tersebut dapat digunakan dalam pendeteksian eDNA C. mccordi dari sampel air. Primer perlu dilakukan optimasi lebih lanjut terkait pendeteksian kelimpahan individu.

.....The Rote snake-necked turtle (Chelodina mccordi) is an Indonesia endemic species with critically endangered and Possibly Extinct in the Wild status. Attempts to reintroduce this turtle have been conducted, however the tracking of C. mccordi individuals in their natural habitat has not been observed. The environmental DNA (eDNA) is an alternative non-invasive method for detection and monitoring of this species. The development of an eDNA method for detection of C. mccordi requires species-specific markers in order to accurately identify the presence of target DNA in the sample. This study aims to develop species-specific primers using the Cytochrome b (Cyt b) as molecular marker for the detection of eDNA from C. mccordi. The specificity and sensitivity of the primers were evaluated using water samples containing C. mccordi and their related species. The extracted eDNA from water samples are then processed using Quantitative Polymerase Chain Reaction (qPCR) and High-Resolution Melting (HRM) techniques. The results showed that the design primer (UI_Cm_Cytb) was successful for detection the presence of C. mccordi from the samples. The sensitivity and specificity values of the primers are relatively high, namely 87.5% and 100%. Futhermore, the primer needs to be optimized and tested regarding the individual abundance in sample.