

Chapter 1

Introduction

1.1 Background

Reptiles have become a prevalent choice of pets for people around the world. The popularity stems naturally from the view that reptiles are unique, low-maintenance, interactive, and rewarding pets. In particular, royal pythons (*Python regius*), also known as ball pythons, have been among the most kept in the past decade (De la Fuente et al., 2023; Valdez, 2021). *P. regius* is categorized as a fairly small snake species, originally collected from three African countries (Togo, Benin, and Ghana). In addition to their size, their docility and care simplicity make them the most traded python species internationally (D’Cruze et al., 2020). To accommodate the global demand and to conserve wild populations, many breeders have established a notably large captive-breeding industry. Ball pythons are relatively easy and cheap to breed, contributing to this phenomenon (Bertocchi et al., 2018; Cacioppo et al., 2021).

Another factor that boosts the ball python's popularity is its phenotypic variability. Over time, wild-caught or captive-produced mutants have been selectively bred to achieve such variations. The term “morphs” refers collectively to monogenic (dominant, incomplete dominant, and recessive) and polygenic ball python traits (Cacioppo et al., 2021; Dao et al., 2023). Aesthetic aside, morphs are desirable among breeders due to their ability to uplift the economic value of individual ball pythons. Morphs located in different loci of the snake’s genome may also be combined to create specific looks, prompting breeders to strategize with their production. To date, the World of Ball Pythons website (<https://www.worldofballpythons.com/>) has recorded more than 7500 single or combination morphs (Bielby et al., 2026).

The recessive mutation “Monsoon” is currently one of the most sought-after and expensive morphs, selling for 20 million rupiahs on the MorphMarket website (<https://www.morphmarket.com/>). Breeders often use the slightly less expensive heterozygous individuals to generate offspring before back-crossing them to produce the homozygous recessive

morph with a lower budget. In accordance with the Mendelian laws of inheritance, first-generation offspring with homozygous wild-type or Monsoon heterozygous alleles will exhibit similar phenotypes (Cacioppo et al., 2021). Breeders usually keep all first-generation offspring to test-breed them for individuals with a copy of the Monsoon allele. This method is both time-consuming and cost-intensive, as sexual maturation of the ball python can last for three years (Ryerson, 2020).

With advances in genetic technology, such as genome sequencing and the polymerase chain reaction (PCR), genetic testing is now possible for various recessive, dominant, and incomplete dominant ball python morphs. A few companies, such as Rare Genetics Incorporated (<https://www.raregeneticsinc.com/>), have offered genetic testing services using snake shed samples (Brown et al., 2022). The testing offers detection of heterozygous and homozygous variants of ball python mutations. This technology helps breeders cut costs and time to select individuals with the desired alleles (Kokiattrakool et al., 2024; Lederer et al., 2023). To date, these services are not available in Indonesia. To offer similar services, it is necessary to conduct a study to design suitable primer pairs for genetic testing. Currently, the primer pairs used by a commercial service that provides the Monsoon morph test are still considered a company trade secret.

This study aims to design primer pairs specific to the Monsoon morph in ball pythons as a preliminary step toward discrimination-based genetic testing in Indonesia. Discrimination-based genetic testing is particularly advantageous for commercial genetic testing, as it does not require identifying specific allele variants when designing primers. Instead, it relies on the visualization of the wild-type, which remains distinct from the mutants, thereby allowing for effective differentiation of the two (Kumsiri et al., 2025).

1.2 Objective

This study aims to identify candidate genes responsible for the Monsoon ball python mutation and to design primer pairs for discrimination-based genetic testing of ball python morph.

1.3 Hypothesis

H_0 : The gene responsible for the ball python morph trait cannot be identified, and no primer pairs can be designed to distinguish them from the mutant variants.

H_1 : The gene responsible for the ball python trait can be identified, and primer pairs can be designed to amplify the gene of interest and used to distinguish variation in the ball python phenotype.