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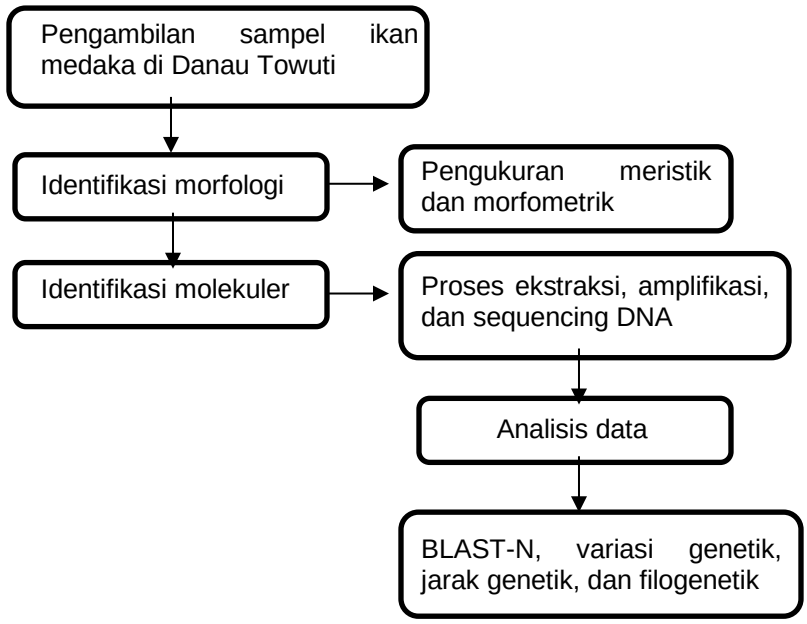
LAMPIRAN

LAMPIRAN

Lampiran 1: Skema Kerja Penelitian



Lampiran 2: Skema Kerja Ekstraksi DNA, Amplifikasi dan Elektroforesis

Proses ekstraksi DNA

- Sebanyak 20 mg jaringan otot sampel ikan medaka yang telah dipreservasi dalam alkohol 96% diambil dari tube sampel
- lalu fillet dihancurkan memakai gunting steril yang telah direndam dengan etanol 70% dan disterilisasi dengan api Bunsen
- setelah itu, sampel dimasukkan ke dalam tube 1,5 ml dan ditambahkan 180 µL larutan buffer ATL serta 20 µL proteinase K 600 mAU/mL, divortex hingga homogen selama 20 detik, kemudian diinkubasi pada suhu 50°C selama 12 jam. Pada 3 jam pertama, sampel divortex setiap 30 menit.
- Tambahkan 200 µl buffer AL dan 200 µL etanol absolut dingin serta vortex selama 20 detik.
- Seluruh campuran di pipet dan dimasukkan ke dalam spin column tube 2 ml, kemudian disentrifugasi 8000 rpm selama 1 menit.
- Cairan collection tube dibuang kemudian pindahkan spin column ke collection tube baru. Tambahkan 500 µl Buffer AW 1, kemudian disentrifugasi 8000 rpm selama 1 menit.
- Cairan dibuang kemudian tempatkan spin column kembali ke collection tube 2 ml. Tambahkan 500 µl Buffer AW 2, kemudian disentrifugasi 13.500 rpm selama 3 menit.
- Cairan collection tube dibuang, lalu spin column dipindahkan ke tube 1,5 ml yang baru dan diinkubasi pada suhu 50°C selama 2 menit
- Lalu tambahkan 250 µL buffer AE, inkubasi pada suhu ruang selama 2 menit, kemudian disentrifugasi 8.000 rpm selama 1 menit. Spin column kemudian dibuang dan tube disimpan sebagai hasil isolasi DNA.

Amplifikasi DNA

- Hasil isolasi DNA diamplifikasi menggunakan primer COI yang terdiri dari forward (FishF2: 5'TCGACTAATCATAAAGATATCGGCAC-3') dan reverse (FishR2: 5'ACTTCAGGGTGACCGAAGAATCAGAA-3').
- Amplifikasi PCR yang digunakan sebanyak 25 µL reaksi per tube yang terdiri dari 12,5 µL Ready Mix PCR 20 (MyTaq™ HS Red Mix Bioline); 1,5 µM tiap primer; 1 mM, MgCl2; 5,5 µL ddH2O steril; dan 5µl DNA. Seluruh larutan tersebut dicampur dalam sebuah PCR tube untuk masing masing sampel dan dimasukkan ke dalam Thermocycler.
- Siklus pada PCR/ *Thermocycler* yang digunakan sebagai berikut: predenaturation pada suhu 95ºC selama 1 menit dengan 1 siklus, selanjutnya denaturation pada suhu 95 ºC selama 15 menit dengan 35 siklus, annealing pada suhu 50ºC selama 30 detik, extension pada suhu 72ºC selama 30 detik, selanjutnya 1 siklus postextension pada suhu 72ºC selama 5 detik, dan hold pada suhu 4ºC

Elektroforesis

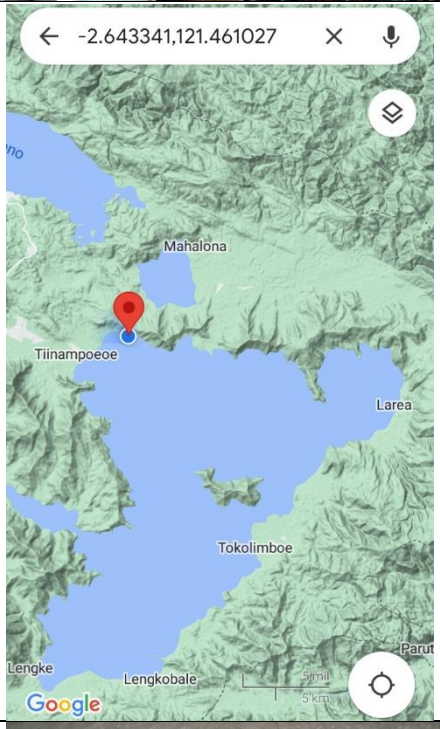
Lampiran 3: Stasiun penelitian

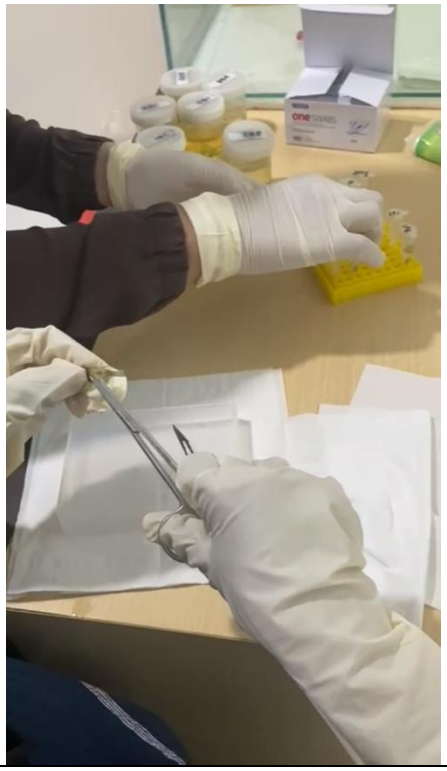
1		Stasiun 1: Tanjung Timbala
2		Stasiun 2: Tanjung Bakara

3		Stasiun 3: Tanjung Saone

Lampiran 4: Pengamatan

1		Suhu
2		pH

3		DO meter
4		GPS
5		Pengukuran morfometrik

6		Pengambilan jaringan otot ikan medaka
7		Sampel ikan medaka

Lampiran 5: Sekuens gen COI ikan medaka dari Danau Towuti

1. Sekuens gen COI 1T1

GGTGCCTGAGCCGGCATGGTAGGAACCGCTTTAAGCCTACTAATCCGCGCTGA
ACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAGATTTACAACGTAATCG
TGACCGCCCACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCGGGGGCGGGGACAGGATGGACGGTTTAT
CCCCCACTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTGTCTCTTCTATTTTAGGTGCCATTAATTTTATT
ACCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAAACGCCGTTAT
TTGTCTGATCAGTACTTATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCGGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGAGACCCCATCCTTTACCAACACTTATTCTGATTCTTC
GGT

2. Sekuens gen COI 1T2

GGTGCCTGAGCCGGCATGGTAGGGACCGCTTTAAGCCTACTAATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAGATTTACAACGTAATC
GTGACCGCCCACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTG
GAGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCT
TTCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCC
TTCTATCCTCCTCCGGCGTAGAGGCGGGGGCGGGGACAGGATGGACGGTTTA
TCCCCCACTAGCAGGAAACCTGGCCCACGCAGGCGCATCCGTAGACCTTACTA
TTTTCTCCCTTCACCTGGCAGGTGTCTCTTCTATTTTAGGTGCCATTAATTTTAT
TACCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAAACGCCGTTA
TTTGTCTGATCAGTACTTATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACT
AGCAGCGGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTT
TGACCCAGCTGGAGGAGGAGACCCCATCCTTTACCAACACTTATTCTGATTCTT
CGGT

3. Sekuens gen COI 1T3

GGTGCCTGAGCCGGCATGGTAGGAACCGCTCTAAGCCTTCTGATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAAATTTATAACGTAATCG
TGACCGCACACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCCGGGGCGGGGACAGGATGAACAGTTTAT
CCTCCGCTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCACTATTATTAACATGAAGCCCCCAACTATCTCCCAATATCAGACGCCGTTATT
TGTCTGATCTGTACTCATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCAGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGGGACCCTATCCTTTACCAACACTTATTCTGATTCTTC
GGT

4. Sekuens gen COI 1T4

GGTGCCTGAGCCGGCATGGTAGGAACCGCTCTAAGCCTTCTGATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAAATTTATAACGTAATCG
TGACCGCACACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG

AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCCGGGGCGGGGACAGGATGAACAGTTTAT
CCCCCGCTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAGACGCCGTTATT
TGTCTGATCTGTACTCATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCAGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGGGACCCTATCCTTTACCAACATTTATTCTGATTCTTC
GGT

5. Sekuens gen COI 2B1
GGTGCCTGAGCCGGCATGGTAGGAACCGCTCTAAGCCTTCTGATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAAATTTATAACGTAATCG
TGACCGCACACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCCGGGGCGGGGACAGGATGAACAGTTTAT
CCCCCGCTAGCAGGGAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAGACGCCGTTATT
TGTCTGATCAGTACTCATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCAGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGGGACCCTATCCTTTACCAACACTTATTCTGATTCTTC
GGT

6. Sekuens gen COI 2B2
GGTGCCTGAGCCGGCATGGTAGGAACCGCTTTAAGCCTACTAATCCGCGCTGA
ACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAGATTTACAACGTAATCG
TGACCGCCCACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCGGGGGCGGGGACAGGATGGACGGTTTAT
CCCCCACTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAAACGCCGTTATT
TGTCTGATCAGTACTTATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCGGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGAGACCCCATCCTTTACCAACACTTATTCTGATTCTTC
GGT

7. Sekuens gen COI 2B3
GGTGCCTGAGCCGGCATGGTAGGAACCGCTCTAAGCCTTCTGATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAAATTTATAACGTAATCG
TGACCGCACACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCCGGGGCGGGGACAGGATGAACAGTTTAT
CCCCCGCTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA

CCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAGACGCCGTTATT
TGTCTGATCTGTACTCATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCAGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGGGACCCTATCCTTTACCAACACTTATTCTGATTCTTC
GGT

8. Sekuens gen COI 2B4

GGTGCCTGAGCCGGCATGGTAGGAACCGCTCTAAGCCTCCTGATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAAATTTATAACGTAATCG
TGACCGCACACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTTCTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCCGGGGCGGGGACAGGATGAACAGTTTAT
CCCCCGCTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCGCTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAGACGCCGTTATT
TGTCTGATCTGTACTCATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCAGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGGGACCCTATCCTTTACCAACACTTATTCTGATTCTTC
GGT

9. Sekuens gen COI 2B5

GGTGCCTGAGCCGGCATGGTAGGAACCGCTCTAAGCCTTCTGATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAAATTTATAACGTAATCG
TGACCGCACACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCCGGGGCGGGGACAGGATGAACAGTTTAT
CCCCCGCTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAGACGCCGTTATT
TGTCTGATCTGTACTCATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTTCTA
GCAGCAGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGAGACCCTATCCTTTACCAACACTTATTCTGATTCTTC
GGT

10. Sekuens gen COI 3S1

GGTGCCTGAGCCGGCATGGTAGGAACCGCTCTAAGCCTTCTGATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAAATTTATAACGTAATCG
TGACCGCACACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCCGGGGCGGGGACAGGATGAACAGTTTAT
CCCCCGCTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAGACGCCGTTATT
TGTCTGATCTGTACTCATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTTCTA
GCAGCAGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGGGACCCTATCCTTTACCAACACTTATTCTGATTCTTC
GGT

11. Sekuens gen COI 3S2

GGTGCCTGAGCCGGCATGGTAGGAACCGCTTTAAGCCTACTAATCCGCGCTGA
ACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAGATTTACAACGTAATCG
TGACCGCCCACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGTTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCGGGGGCGGGGACAGGATGGACGGTTTAT
CCCCCACTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCACTATTATTAACATGAAACCCCCAACTATCTCCCAATATCAAACGCCGTTATT
TGTCTGATCAGTACTTATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCGGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGAGACCCCATCCTTTACCAACACTTATTCTGATTCTTC
GGT

12. Sekuens gen COI 3S3

GGTGCCTGAGCCGGCATGGTAGGAACCGCTCTAAGCCTTCTGATCCGCGCTG
AACTAAGCCAGCCAGGTTCTCTGCTAGGCGACGACCAAATTTATAACGTAATCG
TGACCGCACACGCCTTTGTCATAATCTTTTTTATAGTAATACCTATTATGATTGG
AGGGTTCGGAAACTGATTAGTCCCCCTGATGCTTGGGGCCCCAGACATGGCTT
TCCCACGAATAAACAATATGAGCTTTTGACTACTACCACCCTCTTTCCTCCTCCT
TCTATCCTCCTCCGGCGTAGAGGCCGGGGCGGGGACAGGATGAACAGTTTAT
CCCCCGCTAGCAGGAAACCTGGCCCACGCAGGCGCATCTGTAGACCTTACTAT
TTTCTCCCTTCACCTGGCAGGTATCTCTTCTATTTTAGGTGCCATTAATTTTATTA
CCACTATTATTAACATGAAACCCCCAACTATCTCTCAATATCAGACGCCGTTATT
TGTCTGATCTGTACTCATTACTGCTGTTTTACTCCTTTTATCCCTTCCAGTACTA
GCAGCAGGCATTACAATGCTGCTGACAGATCGAAACCTAAACACAACATTCTTT
GACCCAGCTGGAGGAGGGGACCCTATCCTTTACCAACACTTATTCTGATTCTTC
GGT.