**Supplementary Information S2**

DNA and LR-PCR products were amplified for *CO1* with the primers 5'-CGCCTGTTTAyCAAAAACAT -3' and 5'- GAGTCCGTCdGCnAndGGTTG -3' (1) and for the partial *CYTB* with the primers 5'- TACCATGAGGACArATrTCnTTyTG -3' and 5'- GGrATdGAdCGdAGrATdGCrTAnGC -3'. Partial *ND6–CYTB* of *Quasipaa yei* was amplified with the primers 5'- AACGCAGCACTCTTGTGACC -3' and 5'-GGrATdGAdCGdAGrATdGCrTAnGC-3'. PCR was carried out in a 13.5 µl PCR reaction of 20 ng of DNA, 0.2 µl *taq* DNA Polymerase and 0.1 µl *pfu* (TaKaRa), 0.8 µl 2.5 mM dNTP (TaKaRa), and 0.8 µl 5 µM forward and reverse primers (Invitrogen). PCR was conducted at 94℃ for 5 min, followed by 35 cycles of 94℃ for 30 s, 50℃ for 30 s, and 72℃ for 2 min, and with a final extension at 72℃ for 10 min and holding at 10℃. Sequencing PCR is adding 20 µg of PCR product, 2 µl ABI BigDye3 diluted 6×, and 1.5 µM primer (2 µl) and conducted 25 cycles of 96 ℃ for 10 s, 50℃ for 5 s, and 60℃ for 4 min and holding at 4℃. Sequencing used an ABI-3730 capillary DNA sequencer. The results were assessed by using SeqMan v.7 (DNAStar, WI, USA).

**Supplementary Information S3**

The Ion Torrent platform yields uneven and biased coverage across MtGs as well as nuclear genome (2). We discovered that there are exists amount of small fragments in all the libraries. For example, there are 27.65% (8,538/30,883) reads sequenced incompletely in *Babina adenopleura*. We use the following standard to check sequence incompletion. During synthesis-by-sequencing, extension is from sequence primer across the mtDNA to the end of adapter P1 (Figure S4). When sequence incomplete, generated fragment will be short and do not have the P1 region. Library *Babina adenopleura* had 27.65% reads that do not have a region similar to the P1 adaptor. Such reads involve low-coverage regions that tend to have gaps (Figure S3: purple). This indicated the sequence incomplete is one of the reason result of coverage unevenness. In addition, sequence chip is vital in improving sequence-quality. Difference batches of chips

vary in sequence-quality (3) and we also recommend Ion 316 Chip v2. To reduced coverage unevenness, another sequence platform Illumina is good choice since human MtG was coverage evenness in it (4).

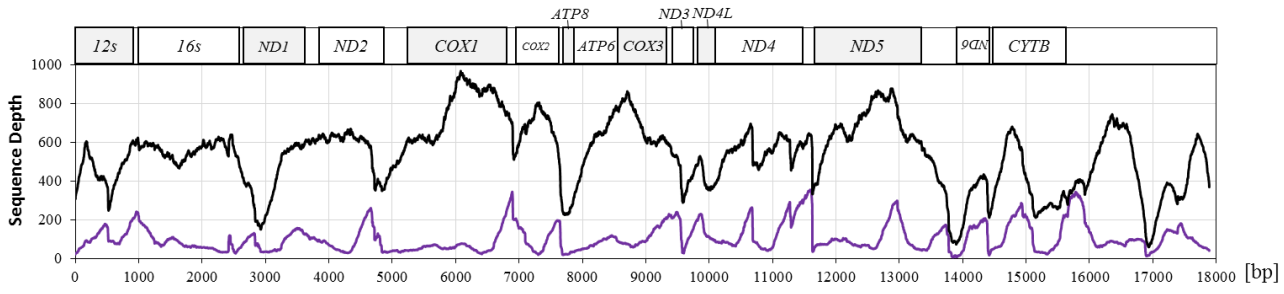


Figure S3. Coverage distributions of *Babina adenopleura* for different read types. Purple line represents coverage using the reads without adaptor sequences; black line showing the overall coverage by using all the reads.

**Supplementary Information S4**

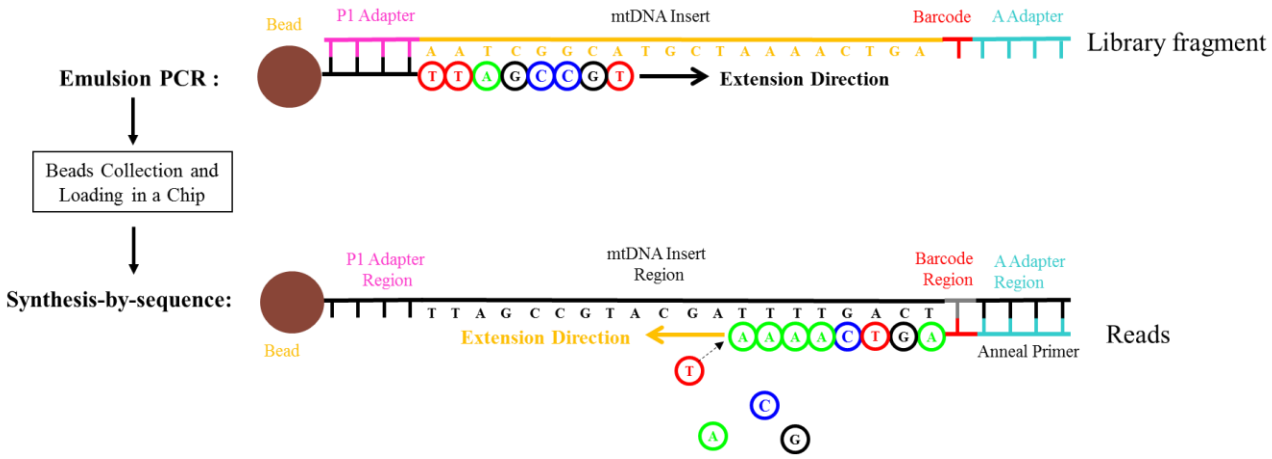


Figure S4. PCR extension during emulsion PCR and Synthesis-by-sequence. In emulsion PCR, the fragment is extend from P1 adapter to end of A adapter according to the library sequence. In the Synthesis-by-sequence, the extend is from A adapter to P1 adapter to get signal base by base at a constant temperature.

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**Supplementary Table S2**

**Table S2.** MtDNA read-distribution and gaps in the library of 33 mixed samples (Samples ranked by read-number).

| Species | Read-number* | Gap position | Reference | Family |
|---|---|---|---|---|
| *Limnonectes bannaensis* | 19267 | Circle | This study | Dicroglossidae |
| *Megophrys palpebralespinosa* | 11315 | Circle | This study | Megophryidae |
| *Leptobrachium ailaonicum* | 6994 | *ND6*: 13990-14016 | This study | Megophryidae |
| *Rhacophorus translineatus* | 6399 | Non-coding region between *ND5* and *12s rRNA* | This study | Rhacophoridae |
| *Kurixalus odontotarsus* | 6354 | Circle | This study | Rhacophoridae |
| *Leptobrachium liui* | 5975 | near *ND6* | This study | Megophryidae |
| *Glandirana tientaiensis* | 5659 | *ND6*: 16006-16090 | NC_025226 | Ranidae |
| *Bufo pageoti* | 5646 | Circle | This study | Bufonidae |
| *Hylarana taipehensis* | 5629 | 1. Non-coding region next to *ND1*: 4503-4542; 2. Non-coding region next to *ND2*: 6426-6463; 3. *ND6*: 14713-14738 | This study | Ranidae |
| *Babina adenopleura* | 5181 | Circle | This study | Ranidae |
| *Kaloula borealis* | 5011 | *ND6*: 13721-13741 | This study | Microhylidae |
| *Leptolalax oshanensis* | 4940 | Circle | This study | Megophryidae |
| *Feihyla vittatus* | 4596 | Almost circle | This study | Rhacophoridae |
| *Occidozyga martensii* | 4254 | Circle | This study | Occidozygidae |
| *Rhacophorus bipunctatus* | 4122 | 1. *ND6*: 13573-13639; 2. Non-coding region between *ND5* and *12s rRNA* | This study | Rhacophoridae |
| *Ichthyophis bannanicus* | 3661 | Circle | This study | Ichthyophiidae |
| *Liurana alpinus* | 3583 | 1. The end of *ND4* and its next non-coding region: 12030-12147, 12148-12377; 2. The front of *ND5*: 12654- | This study | Occidozygidae |

| | | | | |
|---|---|---|---|---|
| | | 12696; 3. *ND6*: 14541-14666 | | |
| *Fejervarya kawamurai* | 2802 | Circle | This study | Dicroglossidae |
| *Raorchestes longchuanensis* | 2518 | Absent *ND5* | This study | Rhacophoridae |
| *Bufo stejnegeri* | 2371 | 1. *ND2*: 4135-4147; 2. *apt6*: 8135-8161; 3. *ND4*: 11245-11257; 4. *ND6*: 13557-13660 | This study | Bufonidae |
| *Leptobrachium chapaense* | 2103 | Almost circle | This study | Megophryidae |
| *Oreolalax xiangchengensis* | 2032 | Circle | This study | Megophryidae |
| *Hyla chinensis* | 1840 | 1. Non-coding region next to *apt8*: 8088-8165 | This study | Hylidae |
| *Scutiger wuguanfui* | 1767 | 1. *ND2*: 4924-4950, 5230-5530; 2. Non-coding region next to *ND5*: 14192-14238; 3. *ND6*: 14445-14474, 14577-14582, 14629-14650 | This study | Megophryidae |
| *Parapelophryne scalpta* | 1690 | 1. Non-coding region next to *ND5*: 12103-12244; 2. Non-coding region next to *ND6*: 14174-14194 | This study | Bufonidae |
| *Bufo tibetanus* | 1434 | 1. *apt6*: 8416-8527; 2. The end of *ND6*: 13787-13922 | NC_020048 | Bufonidae |
| *Pelophylax plancyi* | 1344 | circle | JF730436 | Ranidae |
| *Nanorana maculosa* | 1324 | 1. non-coding region: 2789-2812, 2869-2897; 2. The end of *ND2*: 5202-5292; 3. *COX1*: 6117-6147, 6239-6339 | This study | Dicroglossidae |
| *Bufo gargarizans* | 1118 | 1. *ND6*: 13782-13947; 2. Some gaps in control region | NC_008410 | Bufonidae |
| *Hynobius amjiensis* | 824 | Large gaps | NC_008076 | Hynobiidae |
| *Pachytriton granulosus* | 640 | Large gaps | This study | Salamandridae |
| *Paramesotriton hongkongensis* | 153 | Large gaps | NC_006407 | Salamandridae |
| *Bombina orientalis* | 2* | Large gaps | NC_006689 | Bombinatoridae |