

Figure S1. FISH mapping of microsatellite repeat motifs in *A. kangtingensis* and *A. jinjiangensis*. Four motifs, (AAT)5 (a), (AGAT)5 (b), (AG)10 (c), and (AAC)5 (d), were hybridized in *A. kangtingensis.* Seven motifs, (AAT)5 (e), (AG)10 (f), (AAC)5 (g), (AGAT)5 (h), (AGG)5 (i), (AC)10 (j), and (AT)10 (k), were hybridized in *A. jinjiangensis.* Arrowheads indicate hybridization signals at the terminal or insertion positions of the chromosomes. Chromosomes were counterstained with DAPI (blue). Fluorescent signals of the motifs are shown in red. Bar = 10 μm.

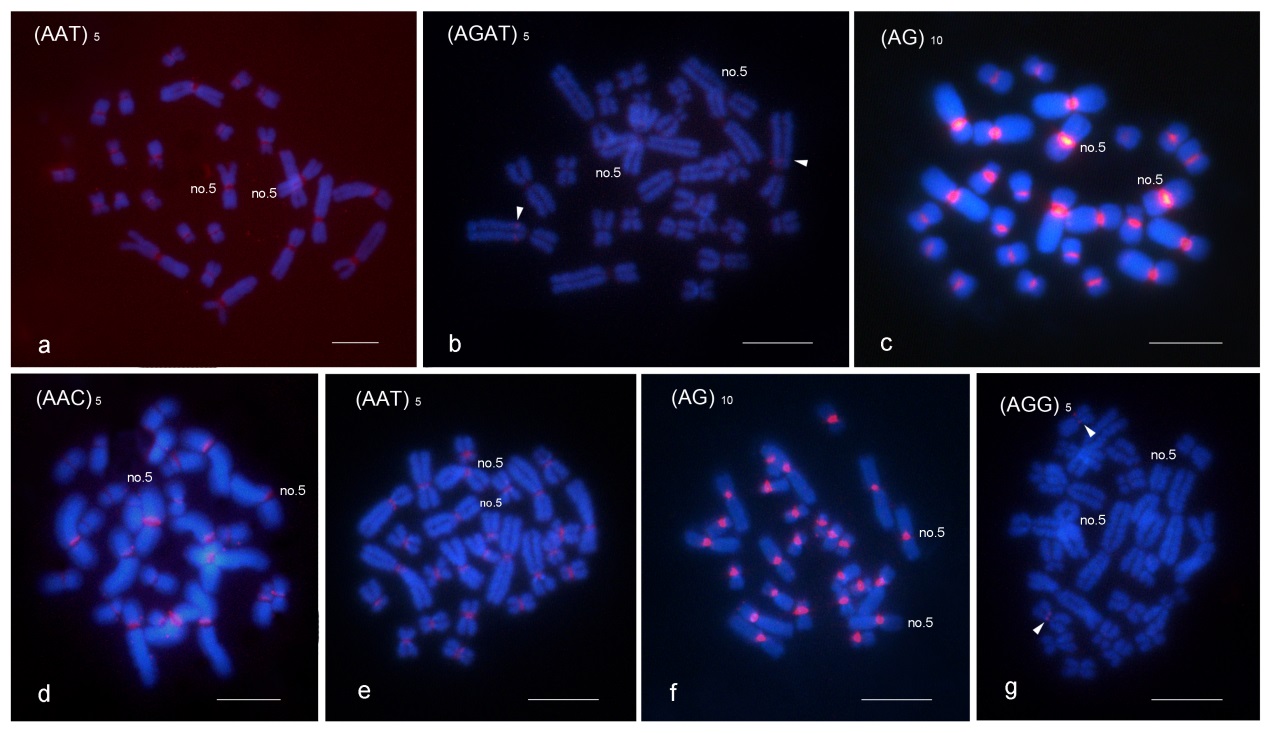


Figure S2. FISH mapping of microsatellite repeat motifs in *A. lifanensis* and *A. loloensis*. Four motifs, (AAT)5 (a), (AGAT)5 (b), (AG)10 (c), and (AAC)5 (d), were hybridized in *A. lifanensis.* Three motifs, (AAT)5 (e), (AG)10 (f), and (AGG)5 (g), were hybridized in *A. loloensis.* Arrowheads indicate hybridization signals at the insertion positions of the chromosomes. Chromosomes were counterstained with DAPI (blue). Fluorescent signals of the motifs are shown in red. Bar = 10 μm.

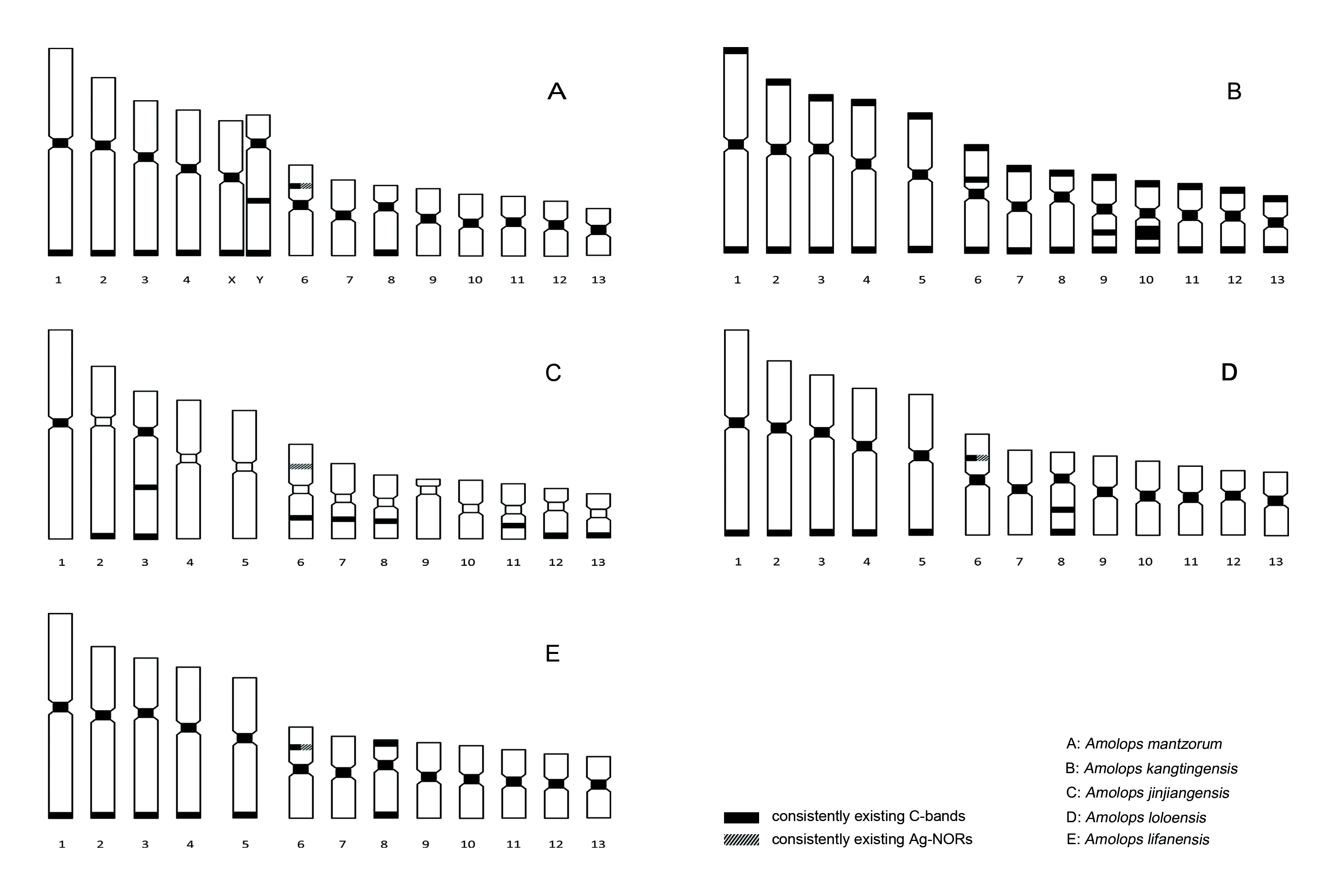


Figure S3. Summary of C-bands and Ag-NORs in five *Amolops* species. The idiogram of C-bands and Ag-NORs in these species is based on Wu et al. (1987a), Wu et al. (1987b), Liu and Yang (1994).The regions where the C-bands and Ag-NORs stained are shown on the chromosomes by different marks: black bar represents consistently present C-bands, grid graph represents consistently present Ag-NORs. A, B, C, D, and E represent *A. mantzorum,* *A. kangtingensis*, *A. jinjiangensis*, *A. loloensis*, and *A. lifanensis* respectively.

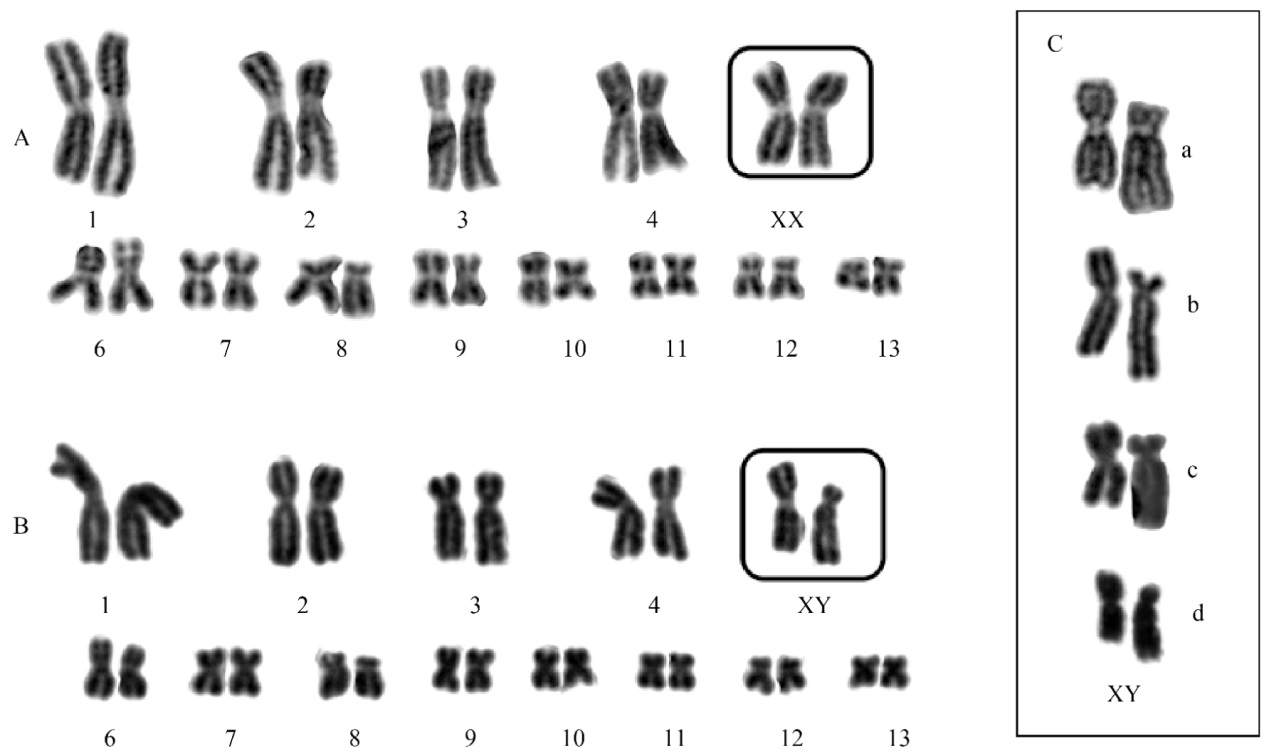
Figure S4. The karyotype of *Amolops mantzorum*. A represents a female from Liangluxiang of Tianquan, B represents a male from Tianba of Luding, C represents chromosome 5 of four males from Xilingxueshan of Dayi(C-a), Dewei of Luding (C-b), Liangluxiang of Tianquan (C-c) and Liuyangcun of Kangding (C-d) (Zhang et al. 2015).

Table S1 Collection sites with the respective sample sizes of *Amolops* species

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| Species | Collection sites | Number of samples |
| *A. mantzorum* | Liangluxiang, Tianquan, Sichuan (N 29°59′28.88″ E 102°22′13.67″) | 1 female, 15 males |
| *A. kangtingensis* | Jiagenbaxiang, Kangding, Sichuan (N 29°47′20.61″ E 101°40′38.78″) | 2 females, 1 male |
| *A. jinjiangensis* | Benzilanzhen, Deqing, Yunnan (N 28°14′31.07″ E 99°17′58.49″)  Nixixiang, Shangri-la, Yunnan (N 28°08′20.67″ E 99°26′5.71″) | 5 females, 1 male  2 males |
| *A. loloensis* | Lajixiang, Yuexi, Sichuan (N 28°25′40.23″ E 102°32′5.86″) | 2 females, 1 male |
| *A. lifanensis* | Shangmengxiang, Lixian, Sichuan (N 31°37′45.72″ E 103°11′15.50″) | 5 males |

Table S2 Chromosome charateristics of *Amolops mantzorum* (Zhang et al. 2015).

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| --- | --- | --- | --- |
| Number | *Amolops mantzorum* | | |
| Relative length | Arm ratio | Type |
| 1 | 13.86±0.99 | 1.16±0.11 | M |
| 2 | 11.85±0.85 | 1.44±0.12 | M |
| 3 | 10.57±0.76 | 1.91±0.13 | SM |
| 4 | 10.09±0.77 | 1.38±0.12 | M |
| 5x | 9.30±0.59 | 1.25±0.13 | M |
| 5y | 9.05±0.47 | 3.29±0.62 | ST |
| 6 | 6.64±0.59 | 1.12±0.12 | M |
| 7 | 5.69±0.59 | 1.12±0.07 | M |
| 8 | 5.37±0.39 | 2.02±0.58 | SM |
| 9 | 5.06±0.30 | 1.15±0.09 | M |
| 10 | 4.71±0.25 | 1.15±0.11 | M |
| 11 | 4.51±0.29 | 1.11±0.11 | M |
| 12 | 4.25±0.31 | 1.22±0.22 | M |