**Supplementary Information**

**Invasive fish retain plasticity of naturally selected, but diverge in sexually selected traits**

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**Supplement 1: Descriptive statistics for phenotypic analyses**

**Table S1**

Summary information of life-history parameters of *G. affinis* from five invasive populations. Presented are means (± SEM) of raw data or EMMs ± SEM, corrected for a standard length of 27.08 mm (female fecundity) and for a stage of embryonic development of 12.23 (embryo lean weight and embryo fat content). Data were obtained from (*a*) wild-caught (*n* = 109 males; *n* = 111 females), (*b*) F1 laboratory-reared (*n* = 108 males; *n* = 111 females) and (*c*) F2 laboratory-reared individuals (*n* = 95 males; *n* = 92 females).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Sex | Population | *N* | Standard length [mm] | Somatic lean weight [mg] | Adult fat content [%] | Fecundity | Investment into reproduction [%] | Embryo lean weight [mg] | Embryo fat content [%] |
|
|
| (*a*) wild-caught fish | ♂ | Ankang | 29 | 19.90 ± 0.38 | 27.24 ± 1.78 | 6.22 ± 0.13 | — | 1.80 ± 0.06 | — | — |
|  | Baoding | 23 | 20.18 ± 0.23 | 26.92 ± 0.93 | 2.84 ± 0.32 | — | 1.91 ± 0.11 | — | — |
|  | Beihai | 19 | 20.14 ± 0.30 | 25.59 ± 1.16 | 2.42 ± 0.20 | — | 2.60 ± 0.11 | — | — |
|  | Chaozhou | 13 | 18.81 ± 0.43 | 18.72 ± 1.39 | 4.00 ± 0.20 | — | 2.93 ± 0.28 | — | — |
|  | Lishui | 25 | 18.33 ± 0.20 | 17.76 ± 0.89 | 5.31 ± 0.17 | — | 2.30 ± 0.12 | — | — |
| ♀ | Ankang | 25 | 28.32 ± 0.68 | 91.82 ± 8.99 | 6.68 ± 0.31 | 35.74 ± 2.49 | 15.57 ± 1.37 | 17.92 ± 2.58 | 2.24 ± 1.18 |
|  | Baoding | 14 | 24.60 ± 0.49 | 63.53 ± 2.07 | 3.12 ± 0.27 | 13.52 ± 3.39 | 5.29 ± 0.73 | 2.38 ± 3.41 | 5.54 ± 1.56 |
|  | Beihai | 25 | 26.90 ± 0.32 | 63.90 ± 2.61 | 3.60 ± 0.30 | 15.17 ± 2.45 | 20.30 ± 0.80 | 17.05 ± 2.62 | 5.14 ± 1.20 |
|  | Chaozhou | 24 | 27.75 ± 0.34 | 63.28 ± 2.56 | 4.46 ± 0.17 | 23.87 ± 2.51 | 20.91 ± 1.52 | 19.72 ± 2.78 | 4.81 ± 1.27 |
|  | Lishui | 23 | 20.84 ± 0.37 | 34.42 ± 1.84 | 5.18 ± 0.40 | 22.67 ± 3.34 | 12.56 ± 0.96 | 5.59 ± 2.67 | 12.02 ± 1.22 |
| (*b*) F1 | ♂ | Ankang | 20 | 23.20 ± 0.33 | 42.35 ± 1.81 | 1.16 ± 0.09 | — | 1.28 ± 0.11 | — | — |
|  | Baoding | 22 | 23.19 ± 0.21 | 47.72 ± 1.70 | 16.87 ± 2.50 | — | 1.41 ± 0.10 | — | — |
|  | Beihai | 26 | 21.38 ± 0.45 | 38.06 ± 2.20 | 1.83 ± 0.26 | — | 1.80 ± 0.16 | — | — |
|  | Chaozhou | 20 | 21.21 ± 0.44 | 32.18 ± 1.98 | 1.32 ± 0.11 | — | 2.38 ± 0.20 | — | — |
|  | Lishui | 20 | 23.97 ± 0.27 | 44.12 ± 1.45 | 14.43 ± 2.63 | — | 2.41 ± 0.08 | — | — |
| ♀ | Ankang | 20 | 27.70 ± 0.33 | 94.71 ± 4.04 | 29.33 ± 2.64 | 38.36 ± 2.75 | 23.71 ± 2.40 | 33.92 ± 2.87 | 18.66 ± 1.31 |
|  | Baoding | 22 | 27.91 ± 0.26 | 103.80 ± 4.36 | 25.75 ± 1.56 | 15.45 ± 2.63 | 15.94 ± 1.61 | 24.22 ± 2.75 | 16.85 ± 1.26 |
|  | Beihai | 29 | 27.87 ± 0.42 | 103.37 ± 4.96 | 21.12 ± 2.56 | 22.55 ± 2.29 | 18.79 ± 1.07 | 25.34 ± 2.41 | 14.71 ± 1.10 |
|  | Chaozhou | 20 | 29.38 ± 0.51 | 109.06 ± 6.43 | 34.49 ± 3.43 | 28.72 ± 2.85 | 20.16 ± 1.82 | 32.63 ± 2.88 | 19.50 ± 1.32 |
|  | Lishui | 20 | 27.33 ± 0.48 | 88.24 ± 5.13 | 28.17 ± 2.33 | 24.74 ± 2.74 | 19.75 ± 3.02 | 26.07 ± 2.83 | 18.65 ± 1.29 |
| (*c*) F2 | ♂ | Ankang | 19 | 18.66 ± 0.57 | 25.67 ± 2.33 | 2.21 ± 0.95 | — | 1.82 ± 0.10 | — | — |
|  | Baoding | 29 | 21.34 ± 0.23 | 36.90 ± 1.36 | 2.86 ± 0.66 | — | 1.74 ± 0.09 | — | — |
|  | Beihai | 13 | 19.88 ± 0.58 | 28.50 ± 2.37 | 1.72 ± 0.15 | — | 1.93 ± 0.11 | — | — |
|  | Chaozhou | 16 | 19.13 ± 0.68 | 26.80 ± 2.90 | 3.29 ± 0.92 | — | 2.09 ± 0.17 | — | — |
|  | Lishui | 18 | 20.96 ± 0.49 | 28.88 ± 2.75 | 8.26 ± 2.02 | — | 3.16 ± 0.19 | — | — |
| ♀ | Ankang | 19 | 27.36 ± 0.49 | 105.00 ± 5.94 | 34.81 ± 1.76 | 13.96 ± 2.82 | 11.65 ± 1.18 | 17.93 ± 2.92 | 23.15 ± 1.34 |
|  | Baoding | 26 | 26.78 ± 0.24 | 93.83 ± 2.77 | 20.90 ± 2.46 | 18.64 ± 2.41 | 16.95 ± 0.91 | 21.87 ± 2.48 | 13.94 ± 1.14 |
|  | Beihai | 15 | 28.12 ± 0.39 | 86.46 ± 4.17 | 27.79 ± 2.51 | 25.24 ± 3.19 | 23.26 ± 1.93 | 26.81 ± 3.27 | 23.41 ± 1.50 |
|  | Chaozhou | 16 | 27.63 ± 0.78 | 84.46 ± 8.08 | 38.45 ± 4.78 | 17.46 ± 3.07 | 16.41 ± 2.66 | 20.23 ± 3.16 | 21.74 ± 1.44 |
|  | Lishui | 16 | 27.56 ± 0.48 | 83.46 ± 5.38 | 32.99 ± 1.22 | 16.73 ± 3.07 | 14.49 ± 1.60 | 18.76 ± 3.14 | 23.06 ± 1.44 |
|  |  |  |  |  |  |  |  |  |  |  |

**Table S2**

Details on relative warps (RWs) retained from our Procrustes analyses on male and female body shape as well as gonopodium shape. Percent variance explained is presented for each RW.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Male body shape | | | Female body shape | | | | Gonopodium shape | | |
| RW | Eigenvalue | % variance explained | | RW | Eigenvalue | % variance explained | RW | Eigenvalue | % variance explained |
| 1 | 0.00059713 | 32.253 | | 1 | 0.00068018 | 36.234 | 1 | 0.00590678 | 33.928 |
| 2 | 0.00029577 | 15.976 | | 2 | 0.00037010 | 19.715 | 2 | 0.00342858 | 19.693 |
| 3 | 0.00016948 | 9.154 | | 3 | 0.00014648 | 7.803 | 3 | 0.00202382 | 11.625 |
| 4 | 0.00012896 | 6.965 | | 4 | 0.00012045 | 6.416 | 4 | 0.00106262 | 6.103 |
| 5 | 0.00011939 | 6.449 | | 5 | 0.00011093 | 5.909 | 5 | 0.00091216 | 5.239 |
| 6 | 0.00010120 | 5.466 | | 6 | 0.00008018 | 4.271 | 6 | 0.00079076 | 4.542 |
| 7 | 0.00008356 | 4.513 | | 7 | 0.00007568 | 4.032 | 7 | 0.00048850 | 2.806 |
| 8 | 0.00006918 | 3.737 | | 8 | 0.00004742 | 2.526 | 8 | 0.00037860 | 2.175 |
| 9 | 0.00005235 | 2.828 | | 9 | 0.00003917 | 2.087 | 9 | 0.00036102 | 2.074 |
| 10 | 0.00004645 | 2.509 | | 10 | 0.00003812 | 2.030 | 10 | 0.00033237 | 1.909 |
| Total | 0.00166347 | 89.850 | | Total | 0.00170871 | 91.023 | Total | 0.01568521 | 90.094 |

**Table S3**

Thermal tolerance limits in *G. affinis* among populations and laboratory generations.Presented are means (±SEM) for upper (CTmax.) and lower (CTmin.) thermal limits. Data were obtained from (*a*) wild-caught (*n* = 77 females), (*b*) F1 laboratory-reared (*n* = 79 females) and (*c*) F2 laboratory-reared individuals (*n* = 68 females). Note that congruent with other studies1,2, CTmax. was less variable than CTmin.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | *N* | CTmax. | *N* | CTmin. |
| (*a*) wild-caught fish | Ankang | 8 | 40.09 ± 0.16 | 8 | 5.05 ± 0.12 |
| Baoding | 8 | 38.16 ± 0.17 | 8 | 8.63 ± 0.21 |
| Beihai | 8 | 39.73 ± 0.06 | 8 | 8.00 ± 0.10 |
| Chaozhou | 8 | 37.58 ± 0.10 | 8 | 7.95 ± 0.05 |
| Lishui | 7 | 36.93 ± 0.12 | 6 | 6.58 ± 0.13 |
| (*b*) F1 | Ankang | 8 | 36.90 ± 0.28 | 8 | 8.15 ± 0.24 |
| Baoding | 8 | 39.56 ± 0.08 | 8 | 6.96 ± 0.49 |
| Beihai | 8 | 37.34 ± 0.07 | 8 | 8.19 ± 0.19 |
| Chaozhou | 8 | 37.41 ± 0.11 | 8 | 7.93 ± 0.30 |
| Lishui | 8 | 37.34 ± 0.13 | 7 | 7.27 ± 0.12 |
| (*c*) F2 | Ankang | 8 | 37.95 ± 0.10 | 8 | 7.13 ± 0.13 |
| Baoding | 6 | 36.73 ± 0.17 | 6 | 7.70 ± 0.13 |
| Beihai | 8 | 37.33 ± 0.11 | 7 | 8.10 ± 0.11 |
| Chaozhou | 8 | 37.24 ± 0.12 | 5 | 7.56 ± 0.13 |
| Lishui | 6 | 36.73 ± 0.17 | 6 | 7.70 ± 0.13 |

**Supplement 2: Additional information on sampling scheme**



**Figure S1**

Sampling sites and morphological landmarks. (*a*) Five sampling sites (Baoding, Ankang, Lishui, Chaozhou, Beihai) across mainland China from which adult *G. affinis* were collected. The map was created using ArcMap 10.2. (*b*) Example of male (upper) and female *G. affinis* (lower) collected in Ankang in April 2016. Dots and numbers demonstrate the 13 landmarks used for morphometric analyses and two additional landmarks (14 and 15) used for the ‘unbending’ procedure in our analyses. (*c*) Photos of a gonopodium showing the 51 landmarks; nomenclature of anal fin rays (3, 4a, 4p, 5a) follows previous work3.

**Supplement 3: Detailed results of phenotypic trait variation**

In the following, we illustrate variation of phenotypic traits for which low ICC-values were uncovered (Table 1), suggesting low broad-sense heritability of the observed population differentiation.



**Figure S2**

Variation in male and female body size. Shown are mean ± SEM (*a*) male and (*b*) female standard length. Black bars represent wild-caught fish, grey bars first-generation laboratory-reared (F1) offspring, and white bars F2 individuals. Intraclass correlation coefficients (ICCs) are presented as an estimate of broad-sense heritability.



**Figure S3**

Visualization of male and female life-history proxies for which low intraclass correlation coefficients (ICCs, shown with associated *P*-values) were uncovered when comparing five populations, as well as wild-caught (black bars), first- (F1, grey bars) and second-generation laboratory-reared individuals (F2, white bars). Shown are EMMs ± SEM, corrected for a body size (SL) of 20.61 mm (males) and 27.12 mm (females), respectively, and for a stage of embryonic development of 12.23 (females). (*a*) Male fat content, (*b*) female somatic lean weight, (*c*) reproductive allocation, (*d*) fecundity, (*e*) embryo lean weight and (*f*) embryo fat content.



**Figure S4**

Components of male body shape (relative warps, RW) for which our analyses revealed low broad-sense heritability estimates (ICC). Shown are EMMs ± SEM, corrected for centroid size and for a body size (SL) of 20.54 mm. Black bars represent wild-caught fish, grey bars F1, and white bars F2 laboratory-reared individuals. Increasing values of morphology-related RWs were associated with (*a*) RW1: more slender bodies, smaller heads, longer caudal peduncles and shorter base length of pectoral fins, (*b*) RW2: decreased width of dorsal fins and a more posterior gonopodium, (*c*) RW3: deeper bodies and blunter heads, (*d*) RW4: more forward-oriented pectoral fins, (*e*) RW5: deeper bodies and smaller heads, (*f*) RW6: sharper heads and larger pectoral fins, (*g*) RW7: decreased width of dorsal fins and longer caudal peduncles, (*h*) RW8: smaller heads and downward-positioned pectoral fins and (*i*) RW10: upward-positioned caudal fins.



**Figure S5**

Components of female body shape (relative warps, RW) for which our analyses revealed low broad-sense heritability estimates (ICC). Shown are EMMs ± SEM, corrected for centroid size and for a body size (SL) of 27.15 mm. Black bars represent wild-caught fish, grey bars F1, and white bars F2 laboratory-reared individuals. Increasing values of morphology-related RWs were associated with (*a*) RW1: larger heads, ventral placement of eyes and longer base length of pectoral fins, (*b*) RW2: more slender bodies and longer caudal peduncles, (*c*) RW3: increased width of dorsal fins, (*d*) RW4: smaller heads and more anterior-positioned pectoral fins, (*e*) RW6: deeper bodies and larger heads, (*f*) RW7: deeper bodies and increased width of dorsal fins, (*g*) RW8: increased width of dorsal fins, (*h*) RW9: decreased width of dorsal fins and (*i*) RW10: larger heads and more posterior pectoral fins.



**Figure S6**

(*a*-*g*) Visualization of components of gonopodium shape differentiation (relative warps, RW) for those seven RWs that yielded low estimates of broad-sense heritability (ICC-values) and (*h*) variation in gonopodium length. We compared wild-caught (black bars), first-generation laboratory-reared (F1, grey bars) and F2 individuals (white bars) from five populations. Shown are EMMs ± SEM, corrected for centroid size and for a gonopodium length of 6.65 mm (gonopodium shape) and in the case of gonopodium length corrected for a standard length of 20.31 mm. Increasing values of morphology-related RWs were associated with (*a*) RW1: closer serrae, closer spines, increased length of the distal element of ray 5a proximal to the curve of the hook and increased length of the elbow on fin ray 4a, (*b*) RW2: slender tips, closer serrae with looser spines, increased length of the elbow on ray 4a, decreased length of the distal element of ray 5a proximal to the curve of the hook and a narrower cavity between fin rays 4a and 4p, (*c*) RW4: larger 4p compound hooks, increased length of the gaps between anal-fin rays 4a and 4p distal to the elbow and decreased length of the elbow on ray 4a, (*d*) RW7: deeper tips with increased dimensions of the cavity between fin rays 4a and 4p, (*e*) RW8: increased length of the distal element of ray 5a proximal to the curve of the hook and increased dimension of the cavity between fin rays 4a and 4p, (*f*) RW9: increased length of the gap between anal-fin rays 4a and 4p distal to the elbow and (*g*) RW10: decreased length of the elbow on ray 4a.



**Figure S7**

Visualization of variation in thermal tolerance limits of female *G. affinis*, which yielded low estimates of broad-sense heritability (ICC-values). Black bars represent wild-caught fish, grey bars F1, and white bars F2 laboratory-reared individuals. Shown are EMMs ± SEM of (*a*) upper and (*b*) lower thermal tolerance limits, corrected for a standard length of (*a*) 27.94 mm and (*b*) 27.85 mm.

**Additional references**

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3. Rosen, D. E. & Gordon, M. Functional anatomy and evolution of male genitalia in poeciliid fshes. *Zoologica* **38**, 1–47 (1953).