

Latitudinal variation in sexual dimorphism in a freshwater fish group

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Tropical animals are characterized by showy ornaments and conspicuous body colours as compared with their temperate relatives. Some recent studies have hypothesized that sexual selection pressures are stronger in the tropics than in the temperate zone. Although negative correlations between latitude and the degree of sexual dimorphism would support this hypothesis, phylogeny should be taken into account in such comparative studies. Comparisons of the degree of sexual dimorphism in body size and fin lengths among species of the Adrianichthyidae, a freshwater fish family having a wide geographical range throughout Southeast and East Asia, revealed that lower latitude species are sexually more dimorphic in all characters than higher latitude species. Phylogenetic generalized least squares analyses using a mitochondrial DNA phylogeny demonstrated that the negative correlations between latitude and the degree of sexual dimorphism become non-significant when phylogeny is considered, but that the variance in the degree of sexual dimorphism is explained not only by phylogeny but also almost equally by latitude. Ancestral state reconstruction indicated that sexual dimorphisms have evolved independently even within major

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clades. These findings are consistent with the view that tropical species are exposed to stronger sexual selection pressures than temperate species. We discuss possible causes of the latitudinal variation in sexual selection pressure.

ADDITIONAL KEYWORDS: Adrianichthyidae – latitude – reproductive seasonality – secondary sexual characteristic – sexual selection – temperate – tropical.

INTRODUCTION

Sexual selection often leads to the evolution of secondary sexual characteristics (e.g. Endler, 1983; Andersson, 1994; Wikelski & Trillmich, 1997; Weatherhead & Dufour, 2005; Husak & Fox, 2008). In polygynous mating systems, sexual selection selects for male traits that are beneficial in fighting for females (intrasexual selection) and/or for being chosen by females (intersexual selection). Therefore, in environments where strong and attractive males can mate with more females, i.e. where sexual selection pressures are high, males will evolve more exaggerated secondary sexual characters. Thus, the degree of secondary sexual characters or the resultant sexual dimorphism has been used as an index of the strength of sexual selection pressures (e.g. Cardillo, 2002; Stuart-Fox & Owens, 2003).

Tropical animals are characterized by showy ornaments and conspicuous body colours as compared with their temperate relatives (e.g. Wallace, 1878; Adams *et al.*, 2014). Naturalists have long sought the evolutionary mechanisms behind this latitudinal variation in the degree of secondary sexual characters or the resultant sexual dimorphism (e.g. Wallace, 1889). Some studies attributed them to latitudinal variation in sexual selection pressure, i.e. tropical species are more exaggerated and sexually more dimorphic, because sexual selection pressures are stronger in tropical regions than in temperate environments (Chui & Doucet, 2009; Painting *et al.*, 2014; Fujimoto *et al.*, 2015). However, this is not a collective view (e.g. Cardillo, 2002; Price *et al.*, 2002; Schemske *et al.*, 2009).

The first and essential step to test whether tropical species are exposed to stronger sexual selection pressures is to find negative correlations between latitude and the degree of sexual dimorphism. In such comparative studies of character evolution, however, phylogeny should be taken into account, because closely related species tend to share ancestral characteristics regardless of the selection pressures to which they are exposed (Felsenstein, 1985; Harvey & Pagel, 1991; Garamszegi, 2014). Such phylogenetic correction is necessary before making any strong inference about latitudinal variation in sexual selection pressure.

The family Adrianichthyidae, commonly referred to as ricefishes or medaka, is known to be sexually dimorphic in body size (e.g. Herder & Chapuis, 2010; Mokodongan *et al.*, 2014), anal- and dorsal-fin lengths (e.g. Kawajiri *et al.*, 2009; Fujimoto *et al.*, 2015) and

body coloration (e.g. Mokodongan *et al.*, 2014; Mandagi *et al.*, 2018), suggesting that these traits are affected by sexual selection. Comparisons of sexual dimorphisms and mating behaviours have demonstrated that high-latitude temperate species/populations are exposed to stronger sexual selection pressures than lower latitude temperate congeners (Fujimoto *et al.*, 2015). However, it has not been tested whether this latitudinal variation can be extended to tropical species, despite the adrianichthyids having a wide geographical range; the family Adrianichthyidae is composed of 37 species in two genera ranging from high-latitude temperate to tropical Asia (Parenti, 2008; Herder & Chapuis, 2010; Magtoon, 2010; Parenti & Hadiaty, 2010; Asai *et al.*, 2011; Herder *et al.*, 2012; Parenti *et al.*, 2013; Mokodongan *et al.*, 2014; Mandagi *et al.*, 2018) (Fig. 1). Thus, this freshwater fish group provides a good model system to explore possible geographical patterns in sexual selection pressures.

In this study, we first compare the degree of sexual dimorphism in body size and fin lengths among almost all adrianichthyids (32 species) throughout Southeast and East Asia and reveal that lower latitude, tropical species are sexually more dimorphic than higher latitude, temperate species. Second, we reconstruct their phylogeny using mitochondrial DNA (mtDNA) sequences and demonstrate that the negative correlations between latitude and the degree of sexual dimorphism become non-significant when phylogeny is considered, but that the variance in the degree of sexual dimorphism is explained not only by phylogeny but also by latitude. We also perform ancestral state reconstruction and reveal that sexual dimorphisms have evolved independently even within major clades. Based on these findings, we argue that tropical species are probably exposed to stronger sexual selection pressures than temperate species. Finally, we discuss possible causes of this latitudinal variation in sexual selection pressure from the viewpoint of reproductive seasonality.

MATERIAL AND METHODS

COMPARISONS OF SEXUAL DIMORPHISM AMONG ADRIANICHTHYIDS

Adrianichthyids are small (~20–190 mm) freshwater or brackish water fishes, having determinate growth

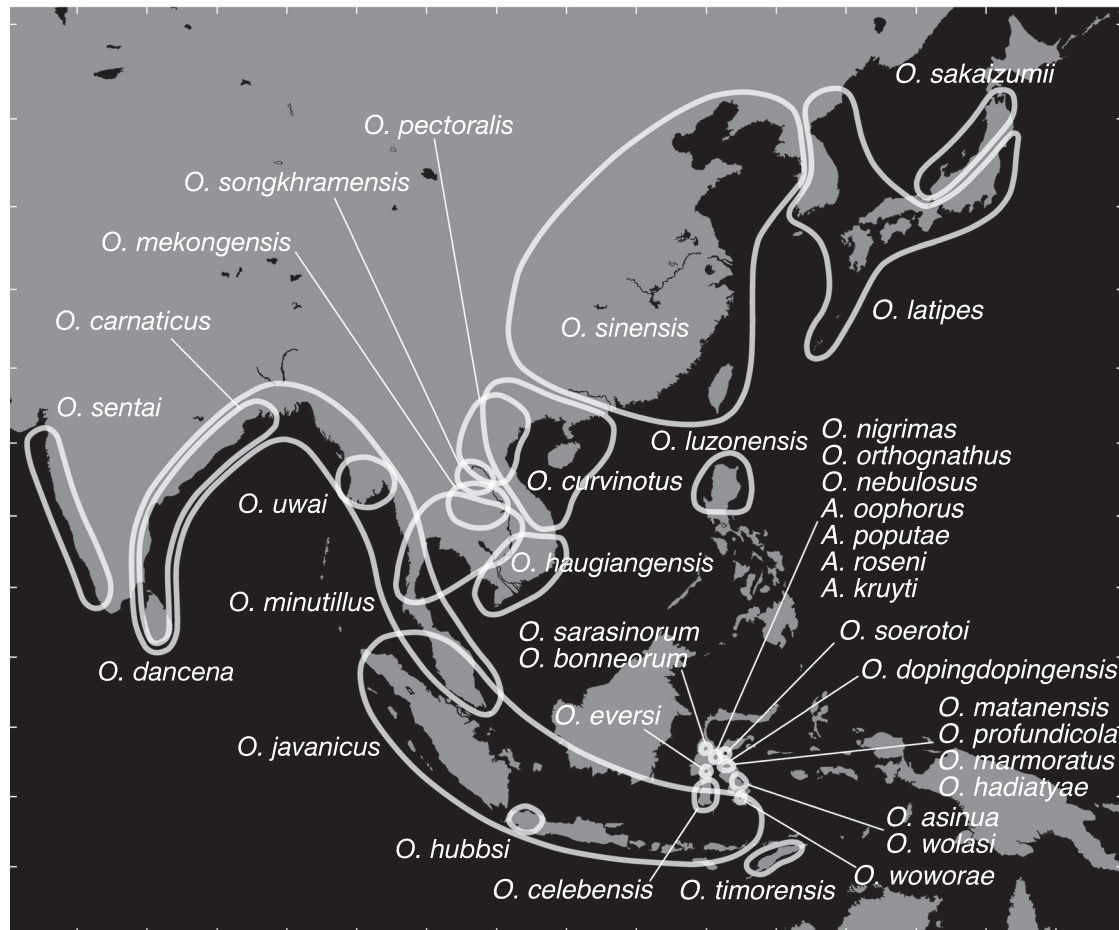


Figure 1. Map showing the geographical ranges of all species of the Adrianichthyidae.

(Iwamatsu, 2006; Parenti, 2008; Kawajiri *et al.*, 2014). Wild individuals or individuals from aquarium strains were obtained for 32 adrianichthyid species found throughout East, Southeast and South Asia (Supporting Information, Table S1). Using a digital camera, we photographed the three largest adult males and three largest adult females of each species (except for only one adult male and one adult female for *Oryzias songkhramensis*), shown in lateral view with a ruler. The digital images were later transferred to a personal computer, and standard length (SL), anal-fin length (AFL) and dorsal-fin length (DFL) were measured for each individual, using image analysis software (Inkscape v.0.92; Free Software Foundation, Inc., Boston, MA, USA). AFL and DFL were defined as the length of the longest anal- and dorsal-fin rays, respectively. The ratios AFL/SL and DFL/SL were calculated for each individual, and the degree of sexual dimorphism in AFL and DFL of each species was defined as the difference in mean AFL/SL and DFL/SL, respectively, between the three males and three females. In addition, the degree of male-biased sexual

size dimorphism (SSD) of each species was defined as the log-transformed ratio of SL of the largest male to the SL of the largest female among the three males and three females. Full details on the comparisons are provided in the Supporting Information Materials and Methods.

PHYLOGENETIC ANALYSIS

The mtDNA phylogeny of the 32 adrianichthyids was estimated using sequences of the NADH dehydrogenase subunit 2 (*ND2*) and/or cytochrome *b* (*cyt b*) genes, with two species of Beloniformes (*Hyporhamphus sajori* and *Cololabis saira*) as outgroups. All sequence data used, except for *ND2* sequences of four *Oryzias* species, *O. hubbsi*, *O. mekongensis*, *O. songkhramensis* and *O. uwai*, were downloaded from the DNA Data Bank of Japan (DDBJ) (Supporting Information, Table S1). For the four *Oryzias* species the *ND2* region was amplified by PCR and then Sanger-sequenced, using the methods and primers described by Mokodongan & Yamahira (2015).

All sequences were aligned separately for *ND2* and *cyt b*, using the ClustalW option in MEGA7 v.7.0.26 (Kumar *et al.*, 2016), and the aligned sequences of *ND2* (1046 bp) and *cyt b* (1141 bp) were concatenated into a single sequence. A maximum-likelihood (ML) phylogeny among these 34 species (32 adrianiichthyids and two outgroups) was estimated using RAxML-NG v.0.9.0 (Kozlov *et al.*, 2019). Full details of the phylogenetic analysis are provided in the [Supporting Information Materials and Methods](#).

TEST OF CORRELATIONS BETWEEN LATITUDE AND SEXUAL DIMORPHISM

To test whether lower latitude species are sexually more dimorphic, Pearson's correlation between the latitude of each species and the degrees of male-biased SSD and sexual dimorphism in fin lengths (AFL/SL and DFL/SL) were calculated, using JMP v.5.1.1 for Macintosh (SAS Institute, Cary, NC, USA). We used the latitude of the site from which each species originated. We also compared the elevation (intercept) of the regression lines between wild-caught species and lab-reared species using analyses of covariance (ANCOVAs) with 'fish origin' as a fixed factor and latitude as a covariate. Moreover, we tested whether each of these correlations persists even when the phylogenetic relationships among species were considered by phylogenetic generalized least squares (PGLS) regression, using the 'pgls' function in the R package 'caper' (Orme, 2013). The PGLS analysis was performed separately for the degree of male-biased SSD and the degree of sexual dimorphism in AFL/SL and DFL/SL, using latitude as an explanatory variable. The mtDNA phylogeny was used as the tree data, and branch lengths were optimized by ML estimations ('lambda' function = ML). Pagel's λ was estimated in each PGLS model. We also estimated the partial coefficient of determinant (partial R^2) of latitude and phylogeny in each PGLS model, using the 'R2' function in the 'rr2' package (Ives & Li, 2018). We used the R^2_{lik} of Ives (2019) as the partial R^2 in this study. Full details of the test of correlations are provided in the [Supporting Information Materials and Methods](#).

ANCESTRAL STATE RECONSTRUCTION OF SEXUAL DIMORPHISM

Ancestral values of the degrees of male-biased SSD and sexual dimorphism in fin lengths (AFL/SL and DFL/SL) at each node in the mtDNA phylogeny were estimated separately, using the 'fastAnc' function in the R package 'phytools' (Revell, 2012). The estimated state was mapped on the phylogeny using the 'contMap' function in phytools.

RESULTS

COMPARISONS OF SEXUAL DIMORPHISM AMONG ADRIANIICHTHYIDS

In the high-latitude species, adult males were smaller than adult females (Fig. 2A). Among the tropical species, males were larger or smaller depending on species, and vice versa in others (Supporting Information, Fig. S1A). The correlation between latitude and the degree of male-biased sexual size-dimorphism was significantly negative ($r = -0.585$, $N = 32$, $P < 0.001$) (Fig. 2A). The effect of fish origin was significant (ANCOVA: $F_{1,29} = 6.181$, $P = 0.019$), indicating that lab-reared species tended to be sexually less dimorphic than wild-caught species. The slope of the regression of the degree of sexual size dimorphism versus latitude was significantly negative (ANCOVA: $F_{1,29} = 10.543$, $P = 0.003$), even when fish origin was incorporated.

Males of tropical species tended to have longer anal and dorsal fins (standardized by SL) than males of temperate species (Supporting Information, Fig. S1B, C). In contrast, females did not show such a latitudinal tendency. Consequently, the correlation between latitude and the degree of sexual dimorphism in fin lengths was significantly negative: lower latitude, tropical species tended to be sexually more dimorphic both in anal-fin length ($r = -0.487$, $N = 32$, $P = 0.005$) (Fig. 2B) and in dorsal-fin length ($r = -0.549$, $N = 32$, $P = 0.001$) (Fig. 2C) as compared with higher latitude species. The effect of fish origin was significant for anal-fin length (ANCOVA: $F_{1,29} = 4.521$, $P = 0.042$), indicating that lab-reared species tended to be sexually less dimorphic than wild-caught species, but it was not significant for dorsal-fin length ($F_{1,29} = 1.364$, $P = 0.252$). The slope of the regression of the degree of sexual dimorphism versus latitude was significantly negative both in anal-fin length (ANCOVA: $F_{1,29} = 5.598$, $P = 0.025$) and in dorsal-fin length ($F_{1,29} = 9.304$, $P = 0.005$), even when fish origin was incorporated.

PHYLOGENY AND TEST OF LATITUDINAL TENDENCY IN SEXUAL DIMORPHISM

The mitochondrial phylogeny revealed that the adrianiichthyids studied were composed of three main clades (Fig. 3), as similarly shown by Takehana *et al.* (2005). The '*latipes* species group' was composed of species distributed mainly in the Indochina Peninsula and East Asia. The three temperate species, *O. sakaizumii*, *O. latipes* and *O. sinensis* (Fig. 1), all belonged to this species group. The '*javanicus* species group' was composed of species distributed throughout Southeast and South Asia. The '*celebensis* species group' was composed of species endemic to Sulawesi and its satellite islands.

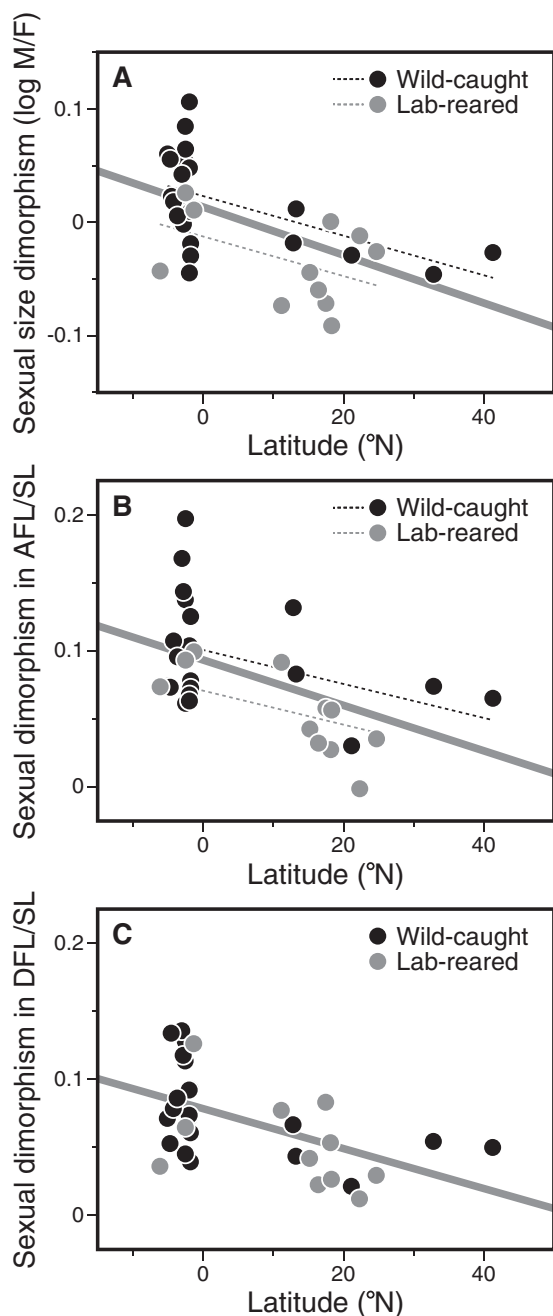


Figure 2. Relationship between latitude and the degree of sexual dimorphism in (A) body size (standard length: SL), (B) anal-fin length (AFL) (standardized by SL) and (C) dorsal-fin length (DFL) (standardized by SL). Plots show means of each species, and the solid line is a single regression line. The dotted lines represent regressions of wild-caught and lab-reared species under the ANCOVA model.

PGLS analysis using latitude as an explanatory variable revealed that all of the negative correlations between latitude and the degree of sexual

dimorphism became non-significant (body size: estimate = $-0.138 \times 10^{-2} \pm 0.074 \times 10^{-2}$ SE, $t = -1.862$, $P = 0.072$; anal-fin length: estimate = $-0.073 \times 10^{-2} \pm 0.071 \times 10^{-2}$ SE, $t = -1.028$, $P = 0.312$; and dorsal-fin length: estimate = $-0.042 \times 10^{-2} \pm 0.060 \times 10^{-2}$ SE, $t = -0.701$, $P = 0.489$). In these PGLS models, Pagel's λ was estimated to be 0.295, 0.301 and 0.494 for the degree of male-biased SSD and the degree of sexual dimorphism in anal-fin length and dorsal-fin length, respectively. Partial R^2 in the PGLS models revealed that phylogeny and latitude almost equally explained the variance in the degree of SSD (partial $R^2 = 0.080$ and 0.091 , respectively) and in the degree of SSD in anal-fin length (partial $R^2 = 0.024$ and 0.019 , respectively). The variance in the degree of sexual dimorphism in dorsal-fin length was explained more by phylogeny (partial $R^2 = 0.181$) than by latitude (partial $R^2 = 0.014$).

ANCESTRAL STATE RECONSTRUCTION OF SEXUAL DIMORPHISM

The ancestral state reconstruction revealed that SSD is male-biased in some species and female-biased in others even within species groups (Fig. 4A). Sexual dimorphism in fin lengths also evolved independently even within the species groups (Fig. 4B, C).

DISCUSSION

EVIDENCE FOR LATITUDINAL VARIATION IN SEXUAL SELECTION PRESSURES

Our morphological analyses of the 32 adrianchthyid species obtained throughout their geographical ranges revealed that male body size and fin lengths increase with decreasing latitude. Consequently, both the degree of male-biased SSD and the degree of sexual dimorphism in fin lengths were negatively correlated with latitude, indicating that tropical species are sexually more dimorphic than temperate species. However, PGLS analyses revealed that the negative correlations between latitude and the degree of sexual dimorphism became non-significant when the phylogenetic relationship among the species was considered. This suggests that the greater (lesser) degree of sexual dimorphisms in tropical (temperate) species may be synapomorphic.

However, Pagel's λ was estimated to be at most ~ 0.5 in the PGLS models, indicating that the variance in the degree of sexual dimorphism cannot be accounted for only by phylogeny. The ancestral state reconstruction also indicated that sexual dimorphisms have evolved independently even within species groups, suggesting that the evolution

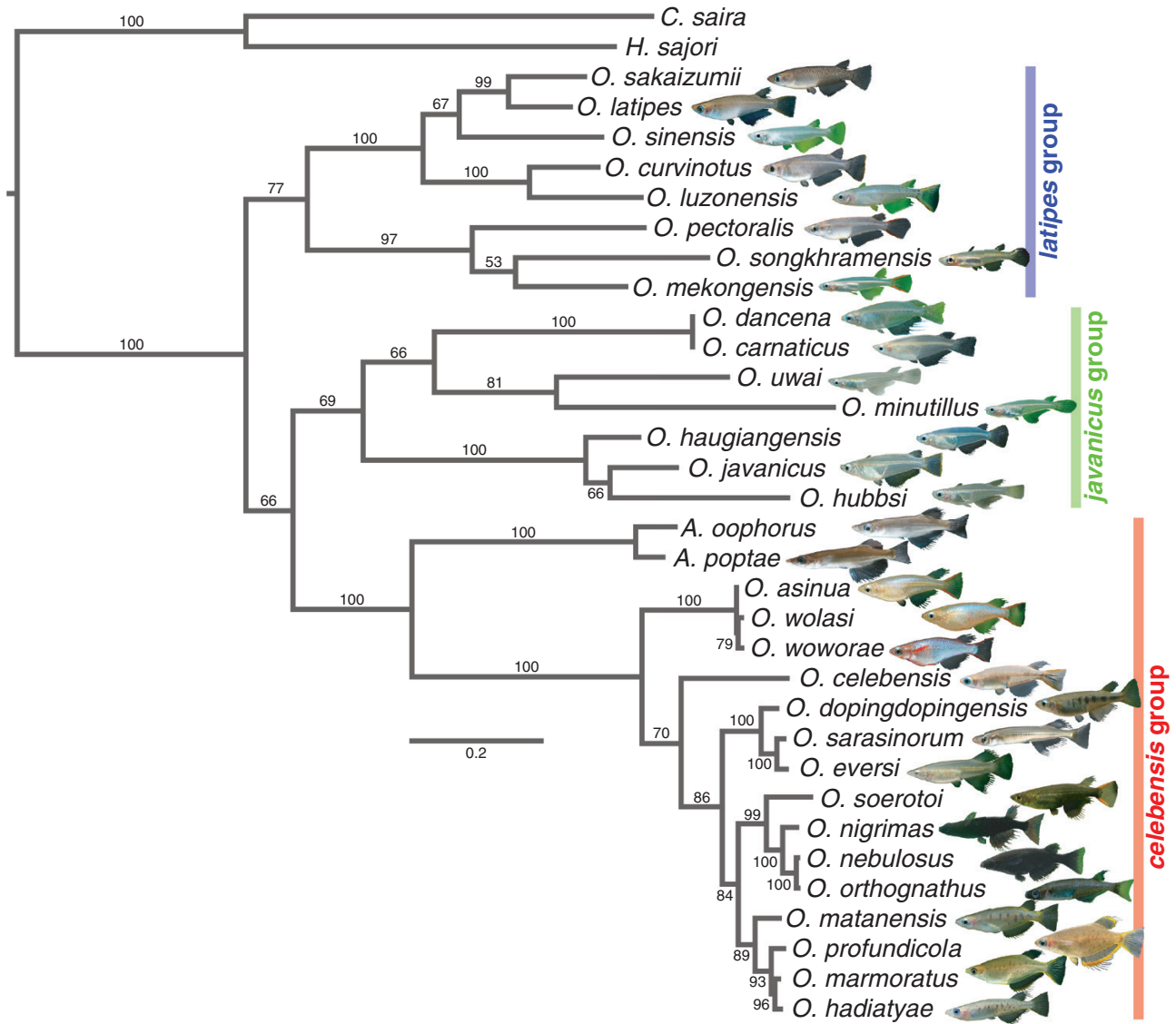


Figure 3. A maximum-likelihood (ML) phylogeny of 32 adrianichthyid species based on mtDNA sequences. Numbers on branches are ML bootstrap values. The scale bar indicates the number of substitutions per site.

of sexual dimorphism is not phylogenetically constrained. Indeed, partial R^2 in the PGLS models revealed that the variance in the degree of SSD and in the degree of sexual dimorphism in anal-fin length is explained not only by phylogeny but also almost equally by latitude. These results indicate that the variance in the degree of sexual dimorphism cannot be explained solely by phylogeny.

It is of note that there was a strong confounding between geographical distributions and phylogenetic positions among the species: that is, all temperate species belong to the 'latipes species group', while species in the 'javanicus species group' and 'celebensis species group' are distributed only in the tropics, which is the main reason why the negative correlations

between latitude and the degree of sexual dimorphism became non-significant in the PGLS models. Although the degree of sexual dimorphism is synapomorphic, this does not mean that it has evolved neutrally (e.g. Freckleton *et al.*, 2002); the greater (lesser) degree of sexual dimorphism in the tropical (temperate) species may have evolved because they are in the tropics (temperate). Regardless, further studies are necessary to resolve this confounding between geographical distributions and phylogeny. One way would be to conduct similar analyses using different taxa having wide geographical ranges; the presence of similar latitudinal patterns in other taxa would support the view that latitude does affect the degree of sexual dimorphism.

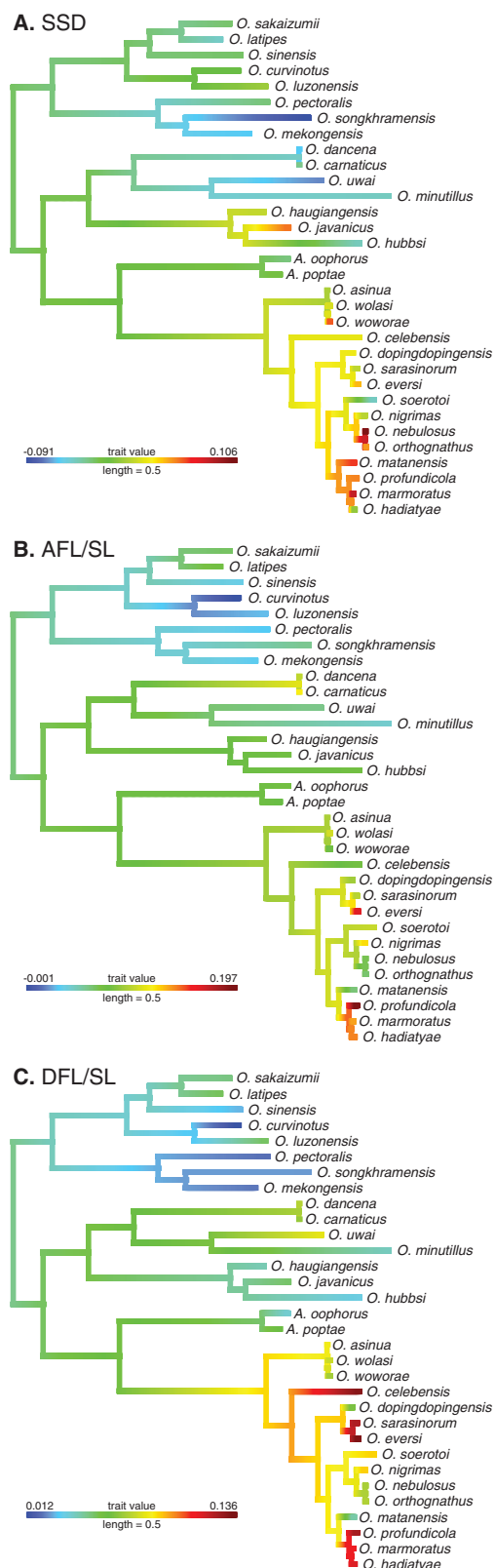


Figure 4. Ancestral state reconstruction of the degree of sexual dimorphism in (A) body size (standard length):

It is also of note that the negative correlation between latitude and sexual dimorphism was found in all characters examined. Our preliminary analysis revealed that the degree of sexual dichromatism, i.e. sexual dimorphism in body coloration, is also negatively correlated with latitude. Previous quantitative trait loci (QTL) mapping with experimental crosses of *O. sakaizumii* and *O. latipes* demonstrated that loci controlling male secondary sex traits, including anal- and dorsal-fin lengths, were different from one trait to another (Kawajiri *et al.*, 2014, 2015), suggesting that these characters could have evolved independently. If secondary sexual characters and the resultant sexual dimorphisms covary among species latitudinally in spite of an absence of genetic correlations, it may be more reasonable to consider that these characters have independently responded to varying sexual selection pressures than to consider that they have evolved neutrally.

We found that lab-reared species tended to be sexually less dimorphic than wild-caught species, suggesting that sexual dimorphisms are phenotypically plastic traits, which may lessen in the laboratory. However, the regression of the degree of sexual dimorphism on latitude was negative, even when 'fish origin' was incorporated, indicating that the observed latitudinal variation has also a genetic basis. Quantitative genetics approaches, such as common-garden experiments (e.g. Falconer, 1989; Conover & Schultz, 1995), are necessary to assess the genetic and environmental variance in the observed latitudinal variation.

CHARACTERS FAVOURED BY INTRA- VS. INTERSEXUAL SELECTION

We found that males are larger than females in many of the tropical species, and vice versa in temperate species. Possibly, this male-biased SSD in tropical species reflects stronger intrasexual selection pressures, because a larger body size is generally beneficial for fighting (e.g. Beaugrand *et al.*, 1996; Passos *et al.*, 2013). Although a larger body size might also be beneficial to males of temperate species, the size benefit might not be as great as that in tropical species, because greater size can also involve potential fitness costs, such as increased vulnerability to starvation or predation (e.g. Lankford *et al.*, 2001; Connoly & Petersen, 2003; Suzuki *et al.*, 2010).

SL) (SSD), (B) anal-fin length (AFL) (standardized by SL) and (C) dorsal-fin length (DFL) (standardized by SL). Estimated changes in the degree of sexual dimorphism on the phylogeny are represented by the branch colour. The scale bar indicates the number of substitutions per site.

Therefore, intrasexual selection pressures probably balance with natural selection pressures at the larger body size in tropical species as compared with that of temperate species.

In contrast, the greater degree of sexual dimorphism in fin lengths and body coloration in many of the tropical species may reflect stronger intersexual selection pressures. [Fujimoto *et al.* \(2014\)](#) demonstrated that males of temperate species (*O. latipes* species complex) with a longer anal fin and/or dorsal fin were less often rejected by females. Mating experiments using *O. woworae*, a tropical species, also revealed that females preferred redder males over less red males ([Ansai, S. *et al.*, unpubl. data](#)), suggesting that the greater degree of sexual dichromatism in tropical species also reflects stronger intersexual selection pressure. However, the role of longer fins in tropical species remains to be examined. Further investigations are necessary to demonstrate the functions of these male traits during mating.

LATITUDINAL VARIATION IN NATURAL SELECTION PRESSURES?

It is theoretically possible that the greater (lesser) degree of sexual dimorphism in tropical (temperate) species reflects evolution in response not to sexual selection but to natural selection. For example, in higher latitude environments where the reproductive season is short, fecundity selection might favour larger females (e.g. [Tarr *et al.*, 2018](#)), which would cause latitudinal variation in female body sizes and resultant SSD. However, this is not the case for adrianiichthyids, because body size as well as anal- and dorsal-fin lengths showed no significant correlation with latitude in females alone.

It is also theoretically possible that latitudinal variation in natural selection pressure indirectly shapes latitudinal variation in sexual dimorphism. It is well known that males in populations exposed to higher predation pressure have evolved less conspicuous ornaments (e.g. [Endler, 1983](#); [Stuart-Fox *et al.*, 2003](#); [Johnson & Candolin, 2017](#)). Therefore, the observed less conspicuous ornaments, such as shorter fins, in males of temperate species could indicate that they are exposed to higher predation pressures than males of tropical species. However, this is probably not the case for adrianiichthyids, because piscivorous predators are apparently more abundant in tropical habitats (such as freshwater halfbeaks of the genera *Dermogenys* and *Nomorhamphus* and freshwater gobies of the genera *Redigobius* and *Glossogobius*; e.g. [Kottelat *et al.*, 1993](#)) than in temperate habitats ([B. K. A. Sumarto *et al.*, pers. observ.](#)). Further studies are needed to assess the relative effect of sexual selection vs. natural selection induced by predators ([Schemske *et al.*, 2009](#); [Roesti](#)

[et al., 2020](#)), and other environmental factors such as water turbidity ([Dugas & Franssen, 2011](#); [Bossu & Near, 2015](#)) and diet quality ([Emlen *et al.*, 2012](#); [Rahman *et al.*, 2013](#)), on the latitudinal variation in sexual dimorphism.

ECOLOGICAL MECHANISM OF THE LATITUDINAL VARIATION IN SEXUAL SELECTION PRESSURE

We have demonstrated here that tropical species are sexually more dimorphic than temperate species, suggesting that the latter are exposed to stronger sexual selection pressure. With that in mind, why might sexual selection pressures vary geographically?

[Machado *et al.* \(2016\)](#) and [Tarr *et al.* \(2018\)](#) considered that sexual selection on males could be higher in warm, aseasonal environments, because when resources and reproductive opportunities are available year round, there may be greater total benefit for males that can control high-quality territories and access to mates. However, the mechanisms behind how males that control territories and mates can benefit more are unclear. On the other hand, [Fujimoto *et al.* \(2015\)](#) proposed that latitudinal clines in reproductive seasonality will lead to latitudinal clines in the operational sex ratio (OSR), which is an important determinant of the strength of sexual selection pressures; biased OSRs cause strong sexual selection, because the more abundant sex, usually males, will compete for available partners ([Emlen & Oring, 1977](#); [Ims, 1988](#); [Janicke & Morrow, 2018](#)). In high-latitude temperate environments, where reproduction is restricted to a favourable season (e.g. [Awaji & Hanyu, 1987](#); [Egami *et al.*, 1988](#); [Isaac, 2005](#)), many mature males and females are expected to appear in synchrony during a short period. Such temporal overlaps in the appearance of mature females will cause OSRs that are more equal, leading to weaker sexual selection pressures. In the tropics, in contrast, a lack of seasonality prolongs reproductive seasons, which will stochastically reduce temporal overlap in the appearance of mature females, while males will tend to mature throughout the year, leading to male-biased OSRs. Our study supports this view that sexual selection pressure is stronger in the tropics than in temperate regions, which potentially explains why tropical animals are characterized by showy ornaments and conspicuous body colours.

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REFERENCES

- Adams JM, Kang C, June-Wells M. 2014.** Are tropical butterflies more colorful? *Ecological Research* **29**: 685–691.
- Andersson M. 1994.** *Sexual selection*. Princeton: Princeton University Press.
- Asai T, Senou H, Hosoya K. 2011.** A new ricefish from northern Japan (Teleostei: Adrianichthyidae). *Ichthyological Exploration Freshwaters* **22**: 289–299.
- Awaji M, Hanyu I. 1987.** Annual reproductive cycle of the wild-type medaka *Oryzias latipes*. *Nippon Suisan Gakkaishi* **53**: 959–965.
- Beaugrand JP, Payette D, Goulet C. 1996.** Conflict outcome in male green swordtail fish dyads (*Xiphophorus helleri*): interaction of body size, prior dominance/subordination experience, and prior residency. *Behaviour* **133**: 303–319.
- Bossu CM, Near TJ. 2015.** Ecological constraint and the evolution of sexual dichromatism in darters. *Evolution* **69**: 1219–1231.
- Cardillo M. 2002.** The life-history basis of latitudinal diversity gradients: how do species traits vary from the poles to the equator? *Journal of Animal Ecology* **71**: 79–87.
- Chui CKS, Doucet SM. 2009.** A test of ecological and sexual selection hypotheses for geographical variation in coloration and morphology of golden-crowned kinglets (*Regulus satrapa*). *Journal of Biogeography* **36**: 1945–1957.
- Connolly PJ, Petersen JH. 2003.** Bigger is not always better for overwintering young-of-year steelhead. *Transactions of the American Fisheries Society* **132**: 262–274.
- Conover DO, Schultz ET. 1995.** Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution* **10**: 248–252.
- Dugas MB, Franssen NR. 2011.** Nuptial coloration of red shiners (*Cyprinella lutrensis*) is more intense in turbid habitats. *Naturwissenschaften* **98**: 247–251.
- Egami N, Terao O, Iwao Y. 1988.** The life span of wild populations of the fish *Oryzias latipes* under natural conditions. *Zoological Science* **5**: 1149–1152.
- Emlen DJ, Warren IA, Johns A, Dworkin I, Lavine LC. 2012.** A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* **337**: 860–864.
- Emlen ST, Oring LW. 1977.** Ecology, sexual selection, and the evolution of mating systems. *Science*, **197**: 215–223.
- Endler JA. 1983.** Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes* **9**: 173–190.
- Falconer DS. 1989.** *Introduction to quantitative genetics, 3rd edn*. New York: John Wiley & Sons.
- Felsenstein J. 1985.** Phylogenies and the comparative method. *The American Naturalist* **125**: 1–15.
- Freckleton RP, Harvey PH, Pagel M. 2002.** Phylogenetic analysis and comparative data: a test and review of evidence. *American Naturalist* **160**: 712–726.
- Fujimoto S, Kawajiri M, Kitano J, Yamahira K. 2014.** Female mate preference for longer fins in medaka. *Zoological Science* **3**: 703–708.
- Fujimoto S, Miyake T, Yamahira K. 2015.** Latitudinal variation in male competitiveness and female choosiness in a fish: are sexual selection pressures stronger at lower latitudes? *Evolutionary Biology* **42**: 75–87.
- Garamszegi LZ. 2014.** *Modern phylogenetic comparative methods and their application in evolutionary biology*. Berlin: Springer-Verlag.
- Harvey PH, Pagel MD. 1991.** *The comparative method in evolutionary biology*. Oxford: Oxford University Press.
- Herder F, Chapuis S. 2010.** *Oryzias hadiatyae*, a new species of ricefish (Atherinomorpha: Beloniformes: Adrianichthyidae) endemic to Lake Masapi, Central Sulawesi, Indonesia. *The Raffles Bulletin of Zoology* **58**: 269–280.
- Herder F, Hadiaty, RK, Nolte AW. 2012.** Pelvic-fin brooding in a new species of riverine ricefish (Atherinomorpha: Beloniformes: Adrianichthyidae) from Tana Toraja, Central Sulawesi, Indonesia. *The Raffles Bulletin of Zoology* **60**: 467–476.
- Husak JF, Fox SF. 2008.** Sexual selection on locomotor performance. *Evolutionary Ecology Research* **10**: 213–228.
- Ims RA. 1988.** The potential for sexual selection in males: effect of sex ratio and spatiotemporal distribution of receptive females. *Evolutionary Ecology* **2**: 338–352.
- Isaac JL. 2005.** Potential causes and life-history consequences of sexual size dimorphism in mammals. *Mammal Review* **35**: 101–115.
- Ives AR. 2019.** R^2 s for correlated data: phylogenetic models, LMMs, and GLMMs. *Systematic Biology* **68**: 234–251.

- Ives AR, Li D. 2018. rr2: An R package to calculate R^2 s for regression models. *The Journal of Open Source Software* **3**: 1028.
- Iwamatsu T. 2006. *The integrated book for the biology of the medaka*. Okayama: Daigaku Kyouiku Publications (in Japanese).
- Janicke T, Morrow EH. 2018. Operational sex ratio predicts the opportunity and direction of sexual selection across animals. *Ecology Letters* **21**: 384–391.
- Johnson S, Candolin U. 2017. Predation cost of a sexual signal in the threespine stickleback. *Behavioral Ecology* **28**: 1160–1165.
- Kawajiri M, Fujimoto S, Yoshida K, Yamahira K, Kitano J. 2015. Genetic architecture of the variation in male-specific ossified processes on the anal fins of Japanese medaka. *G3-Genes Genomes Genetics* **5**: 2875–2884.
- Kawajiri M, Kokita T, Yamahira K. 2009. Heterochronic differences in fin development between latitudinal populations of the medaka *Oryzias latipes* (Actinopterygii: Adrianichthyidae). *Biological Journal of the Linnean Society* **97**: 571–580.
- Kawajiri M, Yoshida K, Fujimoto S, Mokodongan DF, Ravinet M, Kirkpatrick M, Yamahira K, Kitano J. 2014. Ontogenetic stage-specific quantitative trait loci contribute to divergence in developmental trajectories of sexually dimorphic fins between medaka populations. *Molecular Ecology* **23**: 5258–5275.
- Kottelat MA, Whitten J, Kartikasari SN, Wirjoatmodjo S. 1993. *Freshwater fishes of western Indonesia and Sulawesi*. Hong Kong: Periplus Editions.
- Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **35**: 4453–4455.
- Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**: 1870–1874.
- Lankford TE, Billerbeck J, Conover DO. 2001. Evolution of intrinsic growth and energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia menidia*. *Evolution* **55**: 1873–1881.
- Machado G, Buzatto BA, García-Hernández S, Macías-Ordóñez R. 2016. Macroecology of sexual selection: a predictive conceptual framework for large-scale variation in reproductive traits. *The American Naturalist* **188**: S8–S27.
- Magtoon W. 2010. *Oryzias songkramensis*, a new species of ricefish (Beloniformes: Adrianichthyidae) from northeast Thailand and central Laos. *Tropical Natural History* **10**: 107–129.
- Mandagi IF, Mokodongan DF, Tanaka R, Yamahira K. 2018. A new riverine ricefish of the genus *Oryzias* (Beloniformes, Adrianichthyidae) from Malili, central Sulawesi, Indonesia. *Copeia* **106**: 297–304.
- Mokodongan DF, Tanaka R, Yamahira K. 2014. A new ricefish of the genus *Oryzias* (Beloniformes, Adrianichthyidae) from Lake Tiu, Central Sulawesi, Indonesia. *Copeia* **2014**: 561–567.
- Mokodongan DF, Yamahira K. 2015. Origin and intra-island diversification of Sulawesi endemic Adrianichthyidae. *Molecular Phylogenetics and Evolution* **93**: 150–160.
- Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2013. *The caper package: comparative analyses of phylogenetics and evolution in R. R package version 0.5.2*. Available at: <https://CRAN.R-project.org/package=caper>
- Painting CJ, Buckley TR, Holwell GI. 2014. Weapon allometry varies with latitude in the New Zealand giraffe weevil. *Journal of Evolutionary Biology* **27**: 2864–2870.
- Parenti LR. 2008. A phylogenetic analysis and taxonomic revision of ricefishes, *Oryzias* and relatives (Beloniformes, Adrianichthyidae). *Zoological Journal of the Linnean Society* **154**: 494–610.
- Parenti LR, Hadiaty RK. 2010. A new, remarkably colorful, small ricefish of the genus *Oryzias* (Beloniformes, Adrianichthyidae) from Sulawesi, Indonesia. *Copeia* **2010**: 268–273.
- Parenti LR, Hadiaty RK, Lumbantobing D, Herder F. 2013. Two new ricefishes of the genus *Oryzias* (Atherinomorpha: Beloniformes: Adrianichthyidae) augment the endemic freshwater fish fauna of southeastern Sulawesi, Indonesia. *Copeia* **2013**: 403–414.
- Passos C, Tassino B, Loureiro M, Rosenthal GG. 2013. Intra- and intersexual selection on male body size in the annual killifish *Austrolebias charrua*. *Behavioural Processes* **96**: 20–26.
- Price JJ, Lanyon SM, Omland KE. 2002. Losses of female song with changes from tropical to temperate breeding in the new world blackbirds. *Proceedings of the Royal Society B* **276**: 1971–1980.
- Rahman MM, Kelley JL, Evans JP. 2013. Condition-dependent expression of pre- and postcopulatory sexual traits in guppies. *Ecology and Evolution* **3**: 2197–2213.
- Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223.
- Roesti M, Anstett DN, Freeman BG, Lee-Yaw JA, Schluter D, Chavarie L, Rolland J, Holzman R. 2020. Pelagic fish predation is stronger at temperate latitudes than near the equator. *Nature Communications* **11**: 1–7.
- Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology and Systematics* **40**: 245–269.
- Stuart-Fox D, Owens IPF. 2003. Species richness in agamid lizards: chance, body size, sexual selection or ecology? *Journal of Evolutionary Biology* **16**: 659–669.
- Stuart-Fox DM, Moussalli A, Marshall NJ, Owens IPF. 2003. Conspicuous males suffer higher predation risk: visual modelling and experimental evidence from lizards. *Animal Behaviour* **66**: 541–550.
- Suzuki Y, Miyake T, Yamahira K. 2010. An acquisition trade-off with fast growth in a fish, the medaka *Oryzias latipes*: why do low-latitude ectotherms grow more slowly? *Evolutionary Ecology* **4**: 749–759.

- Takehana Y, Naruse K, Sakaizumi M. 2005.** Molecular phylogeny of the medaka fishes genus *Oryzias* (Belontiiformes: Adrianichthyidae) based on nuclear and mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **36**: 417–428.
- Tarr S, Meiri S, Hicks JJ, Algar AC. 2018.** A biogeographic reversal in sexual size dimorphism along a continental temperature gradient. *Ecography* **42**: 706–716.
- Wallace AR. 1878.** *Tropical nature, and other essays*. London: Macmillan.
- Wallace AR. 1889.** *Darwinism: An exposition of the theory of natural selection, with some of its applications*. London: Macmillan.
- Weatherhead PJ, Dufour KW. 2005.** Limits to sexual size dimorphism in red-winged blackbirds: the cost of getting big? *Biological Journal of the Linnean Society* **85**: 353–361.
- Wikelski M, Trillmich F. 1997.** Body size and sexual size dimorphism in marine iguanas fluctuate as a result of opposing natural and sexual selection: an island comparison. *Evolution* **51**: 922–936.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Collection sites of samples used in the morphological analyses, and accession numbers of the *ND2* and *cyt b* gene sequences used in the mtDNA phylogeny. WMA and NBRP represent aquarium strains from the World Medaka Aquarium, Nagoya, Japan, and the National BioResource Project, Okazaki, Japan, respectively.

Figure S1. Relationship between latitude and (A) standard length (SL), (B) anal-fin length (AFL) (standardized by SL) and (C) dorsal-fin length (DFL) (standardized by SL) among the 32 species.