

Stress and sexual signalling: a systematic review and meta-analysis

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3 1 Lay Summary
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7 3 In an analysis of published studies to date, we found that physiological
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10 4 measures of stress were not associated with the expression of traits used to attract
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12 5 mates. We found, however, that stress had a detrimental effect on opposite sex
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14 6 preferences, so that individuals with physiological evidence of high stress were less
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16 7 attractive to the opposite sex. This suggests that stress may mediate attractiveness to
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18 8 the opposite sex, but that we do not yet know which physical or behavioural traits
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20 9 signal stress.
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10 29 Title: Stress and sexual signalling: a systematic review and meta-analysis11
12 3013
14 31 Short title: Stress and sexual signalling15
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18 33 Abstract19
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23 35 The vertebrate stress response has been shown to suppress investment in
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25 36 reproductive and immune function, and may also lead to a reduced investment in the
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27 37 production of secondary sexual traits. However, it has been difficult to model roles of
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29 38 stress in sexual selection due to the inconsistent results seen in empirical studies
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31 39 testing for the effect of stress on the expression of secondary sexual traits. We
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33 40 conducted a phylogenetically-controlled meta-analysis of published associations
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35 41 between physiological correlates of stress and sexual signalling in vertebrates in order
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37 42 to identify any consistent patterns. Our analysis included signalling in both males and
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39 43 females, four stress measures, and four categories of sexually selected traits
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41 44 (vocalisations, traits that varied in size, traits that varied in colouration, and opposite
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43 45 sex preference). Across 38 studies of 26 species there was no significant relationship
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45 46 between physiological correlates of stress and the expression of sexual signals. Mean
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47 47 effect size, however, varied significantly across the four types of sexually-selected
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49 48 trait. We propose development of a model which incorporates the nuanced effects of
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51 49 species ecology, trait type, ecological context and the complex nature of the
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53 50 physiological stress response, on the expression of sexually-selected traits.
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14 56 There is a growing movement towards incorporating the organising role of
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16 57 stress (i.e. conditions where environmental demands exceed an organism's regulatory
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18 58 capacity; Koolhaas et al. 2011) on the allocation of somatic resources, into life history
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20 59 models of behaviour (Buchanan 2000; Korte et al. 2005; Husak and Moore 2008;
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22 60 Bonier et al. 2009; Moore and Hopkins 2009). The vertebrate stress response, for
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24 61 example, includes adaptive activation of the hypothalamic-pituitary-adrenal axis
25
26 62 culminating in the release of glucocorticoids (GCs), which divert resources away from
27
28 63 long term functions and into short term priorities (Cote et al. 2006). While this
29
30 64 promotes survival in the short term, chronically elevated GCs suppress reproduction
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32 65 (Sapolsky et al. 2000) and immune function (Martin 2009). GCs, then, may mediate
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34 66 the relationship between the environment and behavioural trade-offs.

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38 67 The potential for stress to influence the expression of secondary sexual traits
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40 68 has long been recognised by ecologists (e.g. Buchanan 2000). Originally, GCs were
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42 69 predicted to influence sexual signals indirectly via effects on the immune system
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44 70 (Møller 1995; Buchanan 2000) either independently or in interaction with testosterone
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46 71 (Buchanan 2000; Roberts et al. 2007; Husak and Moore 2008). While testosterone has
47
48 72 received most attention to date, it does not account for complexity in the cross-species
49
50 73 data, and GCs have been proposed to interact with the sex hormone in effects on
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52 74 secondary sexual traits (Roberts et al. 2004). More recently, the physiological stress
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54 75 response itself has been proposed to be under sexual selection, such that secondary
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3 76 sexual traits provide cues to individual differences in, for example, stress reactivity or
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5 77 the efficiency of negative feedback (Pfaff et al. 2007; Roberts et al. 2007; Husak and
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7 78 Moore 2008; Bortolotti et al. 2009; Schmidt et al. 2012). Finally, GCs have been
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9
10 79 proposed to influence secondary-sexual traits indirectly via effects on body condition
11
12 80 (Husak and Moore 2008).

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14 81 At first glance, empirical evidence for effects of stress on the expression of
15
16 82 signals used to attract the opposite sex is inconsistent, with some studies reporting
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18 83 detrimental effects of physiological proxies of stress (e.g. Douglas et al. 2009), others
19
20 84 an enhancing effect (e.g. Fitze et al. 2009) and some reporting no relationship (e.g.
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22 85 Setchell et al. 2010). Meta-analysis is well suited to determining common effects
23
24 86 across a range of study systems, especially when empirical results are mixed and
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26 87 many studies may report non-significant results due to low statistical power (Arnqvist
27
28 88 and Wooster 1995; Koricheva et al. 2013). Meta-analysis also allows us, sample size
29
30 89 permitting, to investigate potential moderators of effect size which may generate such
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32 90 inconsistent results (Jennions et al. 2012; Koricheva et al. 2013). We thus performed a
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34 91 phylogenetically-controlled meta-analysis of published studies in which the effect of
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36 92 physiological proxies of stress on the expression of secondary sexual traits was
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38 93 reported. Our first aim was to crystallise any consistent relationships between proxies
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40 94 of stress and the expression of traits across species in order to determine which, if
41
42 95 any, of the proposed roles of stress are best supported by the data. In addition, we
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44 96 analysed 4 potential sources of variation in the observed effect sizes: (a) the sex of the
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46 97 signaller, (b) the measure of stress; (c) the type of signal; and (d) taxonomic group.
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99 METHODS

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101 (a) Literature search

102 We conducted a systematic review of studies published up to November 2014
103 concerning the relationship between stress and secondary sexual traits. We followed
104 the PRISMA protocol for conducting systematic reviews (Moher et al. 2009,
105 Nakagawa and Poulin, 2012). On November 22nd 2014, we searched for the following
106 keywords using the TOPIC field in Web of Science ('stress' OR 'glucocorticoid' OR
107 'corticoster*') AND ('sexual trait' OR 'sexual selection' OR 'sexual signal*' OR
108 'mate choice' OR 'attracti*'). We also contacted authors of relevant publications to
109 identify any additional records. The number of records obtained from each of these
110 approaches is given in the supplementary material. In Figure 1 we present a PRISMA
111 flow diagram showing the number of records obtained from our searches, and the
112 number of records excuded following the application of our selection criteria outlined
113 below.

114

115 [Figure 1 about here]

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117 (b) Criteria fot study inclusion

118 We only included those studies in which the following criteria were met: (1)
119 subjects were adults; (2) subject sex was specified; (3) physiological indices of stress
120 were measured; (4) there was sufficient statistical information to calculate an effect
121 size (either in the publication, or provided by the author). We excluded 13 studies
122 which did not meet these criteria, as well as a subset of results from 1 further study
123 (see Figure 1 and Table S1). This yielded a sample of 118 results from 38 studies of
124 26 species (for all effect sizes see Table S3). We included data concerning both males

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3 125 and females. We obtained effect sizes from four vertebrate classes: amphibians,
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5 126 reptiles, birds and mammals.
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8 127 Four categories of stress measurement were reported: baseline GCs, peak or
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10 128 total GCs produced in response to a stressor, experimental elevation of GCs, and
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12 129 long-term stress. Baseline GCs were typically measured within 3 – 5 minutes of
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14 130 capture (e.g. Douglas et al. 2009). Experimental elevation of GCs up to 4 times above
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16 131 baseline was achieved via subcutaneous implants containing GCs (e.g. San Jose and
17
18 132 Fitze 2013). Long-term stress was assessed in three ways: GCs deposited in feathers,
19
20 133 faeces or hair; the ratio of heterophils to lymphocytes (a white blood cell count that
21
22 134 correlates with baseline GCs; Vleck et al. 2000); and the expression of heat shock
23
24 135 proteins (highly conserved proteins that are elevated under stress; Sørensen et al.
25
26 136 2003). Both heterophil-to-lymphocyte ratio (Davis et al. 2008) and heat shock
27
28 137 proteins (Sørensen et al. 2003) are widely used as proxies of recent and long-term
29
30 138 stress in the ecological literature.
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34 139 The effect sizes we obtained considered a wide range of secondary sexual
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36 140 traits, which we sorted into four categories: colouration, vocalisation, morphological
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38 141 traits, and opposite sex preferences. The colouration category included examples in
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40 142 birds, mammals and reptiles. The amount of colouration was measured in several
41
42 143 different ways, including: brightness, hue, saturation, proportion of structure (e.g. eye
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44 144 ring) that is pigmented, UV reflectance and colour reflectance. The vocalisation
45
46 145 category included singing in birds, and calling in amphibians and a mammal species
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48 146 (rock hyrax *Procavia capensis*). The parameters measured varied according to the
49
50 147 nature of vocalisation in each species, and included song rate, complexity and
51
52 148 repertoire size in birds; the latency to call, call duration, call rate and vocal effort in
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54 149 amphibians; and whether calling/singing was observed or not (rock hyrax,
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3 150 amphibians). Effect sizes included in the morphological trait category all considered
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5 151 bird species, and assessed the size of secondary sexual characters, such as comb or tail
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7 152 length. We also included in this category effect sizes considering the size of a
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10 153 coloured structure (but not the colouration itself), such as epaulet size in the red-
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12 154 winged blackbird (*Agelaius phoeniceus*). Finally, while not a secondary sexual trait
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14 155 per se, opposite sex preference was included as an indirect measure of the level of
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16 156 sexual signalling, with the assumption being that attractiveness to the opposite sex is a
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18 157 function of investment in secondary sexual traits. We rely on author judgements
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21 158 regarding whether each trait is a secondary sexual trait or not. For full coding of effect
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23 159 sizes for each moderator variable see Table S3.
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27 161 (c) Effect sizes

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29 162 We used Pearson's product moment correlation coefficient (r) as the measure
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31 163 of effect size as it was easily computable from statistical information included in most
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33 164 of the studies returned by the systematic review, and is an intuitive measure of effect
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35 165 size that is widely used in meta-analysis (Rosenthal 1991). Here, r represented the
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37 166 magnitude of an association between a physiological index of stress and the
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39 167 expression of a secondary sexual trait, or of a difference in expression of a secondary
40
41 168 sexual trait between individuals exposed to exogenous GCs and controls. If studies
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43 169 did not report r , it was computed from the available statistical information or from
44
45 170 additional information provided by the author using using the Practical Meta-Analysis
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47 171 Effect Size Calculator (<http://www.campbellcollaboration.org>) following established
48
49 172 methods (e.g. Rosenthal 1991). Table S2 gives full details on the calculation of effect
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51 173 sizes when r was not reported. If multiple valid effect sizes were presented for a given
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53 174 study we included them all, and controlled for the possible non-independence between
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3 175 effect sizes arising from this by including study ID as a random effect in all models
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5 176 (see below).

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7 177 Before performing the analysis, all effect sizes were converted using Fisher's
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9 178 z-transform of the correlation coefficient (Zr), which has more desirable properties
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11 179 than r when approaching ± 1 (Koricheva et al. 2013). All models were run using Zr .
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13 180 Mean effect size estimates derived from the models were then converted back to r for
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15 181 presentation. The associated variance for each effect size was calculated as $1/(n - 3)$
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17 182 (Borenstein et al. 2009).
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22 23 184 (d) Phylogeny

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25 185 Recent developments in meta-analysis have allowed researchers to control for
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27 186 the potential non-independence of effect sizes due to phylogenetic history, by
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29 187 incorporating phylogenetic relatedness as a random factor in meta-analysis models
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31 188 (Hadfield and Nakagawa, 2010). This can be done even when accurate branch length
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33 189 data is lacking. As there is no single phylogeny available for all species included in
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35 190 the analysis, we constructed a supertree by combining multiple trees from several
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37 191 different sources. We used taxonomic groupings in cases where phylogenetic data
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39 192 were not available for species in our sample (Hadfield and Nakagawa, 2010). We
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41 193 obtained phylogenetic trees from several sources. For the basal relationships among
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43 194 tetrapods we used Xia et al. (2003). For the relationships among amphibians we used
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45 195 Pyron and Wiens (2011). For the relationships among mammals we used Murphy et
46
47 196 al. (2001). For the relationships among birds we used Hackett et al. (2008) and
48
49 197 Ericson et al. (2006), with trees created using the online tool (birdtree.org)
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51 198 accompanying Jetz et al. (2012).
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3 199 As branch length data were not available for this phylogeny, we first set all
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5 200 branch lengths to one. The tree was then transformed to make all tips
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7 201 contemporaneous using FigTree v1.4 using the cladogram option. Thus total branch
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9 202 length was determined based on the total number of nodes in the tree. The final tree
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11 203 can be seen in Figure 2. Note that branch lengths are likely underestimated for
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13 204 distantly related lineages, and overestimated for lineages containing several species
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15 205 (e.g. *Ficedula*).
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21 207 [Figure 2 about here]
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24 25 209 (e) Meta-analysis

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27 210 We implemented multilevel meta-analyses using a Bayesian linear mixed-
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29 211 effect model approach. Multilevel meta-analytic models are random-effects models
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31 212 (see Borenstein et al. 2009) incorporating additional random factors (following
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33 213 Nakagawa and Santos 2012). This allowed us to control for three potential sources of
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35 214 non-independence in our data set. In several cases we obtained multiple effect sizes
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37 215 from a single study, and from different studies testing a single species. We controlled
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39 216 for this by including study ID and species ID as random effects in all models. Non-
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41 217 independence in effect sizes may also arise due to phylogenetic inertia, so that the
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43 218 relationship between stress and secondary sexual trait expression is more similar for
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45 219 closely related species (Hadfield and Nakagawa, 2010; Koricheva et al. 2013).
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47 220 Phylogeny was thus included as a random effect by incorporating the phylogenetic
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49 221 tree shown above. All the models presented included study ID, species ID and
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51 222 phylogeny as random factors.
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3 223 Meta-analysis models were implemented using the MCMCglmm function
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5 224 from the package *MCMCglmm* (Hadfield 2010). Details on MCMCglmm model
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7 225 specification and testing are presented in the supplementary material. All results
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9 226 presented are based on models fitted using an inverse gamma prior for all random
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11 227 effects and residuals (following Lim et al. 2014). We first ran an intercept-only model
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13
14 228 to determine the mean effect size across all studies. We present our results as mean
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16 229 posterior estimates of r (back-converted from Zr after analysis), and consider a mean
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18 230 estimate to be significantly different from zero if the highest posterior density interval
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21 231 (LHPD to UHPD, also known as the 95% credible interval) does not overlap zero.

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23 232 We assessed the total level of heterogeneity among effect sizes using a
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25 233 modified version of the I^2 statistic (Higgins et al. 2003), following Nakagawa and
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27 234 Santos (2012). The original I^2 statistic describes the percentage of total variation in
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29 235 effect sizes that is due to heterogeneity rather than chance (Higgins et al. 2003).
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31
32 236 However this statistic has to be modified when additional random effects are included
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34 237 in the model. This method can also be used to partition total heterogeneity into that
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36 238 associated with each of the random effects in the model (Nakagawa and Santos 2012).
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38 239 In other words, this allows us to assess the percentage variance in effect size
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41 240 explained by the different random effects (Lim et al. 2014). Substantial residual
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43 241 heterogeneity remaining after accounting for the random effects indicates that there
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45 242 may be further factors influencing effect size that are not included in the model. We
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47 243 follow Higgins et al. (2003) in considering I^2 values of 25%, 5% and 75% as
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49 244 representing small, medium and large amounts of heterogeneity respectively.

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51 245 The intercept-only model indicated significant heterogeneity in effect sizes
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53 246 even after variance associated with the three random factors was accounted for, and so
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56 247 we next investigated potential moderators of mean effect size using a model-selection
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3 248 approach (Nakagawa and Santos 2012). We performed a series of meta-regression
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5 249 models, each of which included study ID, species ID, and phylogeny as random
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7 250 effects, and one or more categorical fixed effects. Model fit was determined using the
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9 251 deviance information criterion (DIC), which is a Bayesian equivalent of traditional
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11 252 information theoretic criteria, and a change in DIC of 2 or more was considered to
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13 253 significantly improve model fit (Spiegelhalter et al. 2002). Finally, we used a separate
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15 254 meta-regression model (minus the intercept) for each categorical fixed effect
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17 255 (taxonomic class, sex, stress measure and trait type) to estimate the mean effect size
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19 256 for each factor level. Each model included study ID, species ID and phylogeny as
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21 257 random effects.
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25 258 We looked for signs of two types of publication bias in our dataset. First, we
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27 259 tested for a bias associated with the failure to publish non-significant or positive
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29 260 results (Koricheva et al. 2013) in two ways. We tested for a relationship between
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31 261 effect size and study precision ($1/SE$) using linear regression (Egger et al. 1997). Due
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33 262 to the potential non-independence of effect sizes in our dataset (due to being
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35 263 measured in the same study or species, or due to shared ancestry) we used residual
36
37 264 effect size, as residuals are theoretically independent of each other (Nakagawa and
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39 265 Santos 2012). We also performed a trim-and-fill analysis using the package *Metafor*
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41 266 (Viechtbauer 2010). This test explicitly searches for asymmetry in the funnel plot
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43 267 (showing the relationship between effect sizes and a measure of their variance), which
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45 268 is assumed to reflect publication bias (Duval and Tweedie 2000). The trim-and-fill
46
47 269 function then imputes “missing” effect sizes until the funnel plot is symmetrical, and
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49 270 then gives a new effect size estimate from a meta-analysis model including these new
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51 271 effect sizes (Duval and Tweedie 2000). Again due to non-independence of effect sizes
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53 272 this analysis was performed on the residuals (Nakagawa and Santos 2012). The
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3 273 difference in mean effect size estimated from this analysis was then used to adjust the
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5 274 original mean effect size (and associated HPD interval) from the intercept-only
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7 275 model. Second, we assessed whether there was any temporal trend in mean effect size
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10 276 by testing for the rank correlation between effect size and year of publication
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12 277 (Koricheva et al. 2013). A significant temporal trend could reflect publication bias if
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14 278 for example studies showing non-significant effects are less likely to be published
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16 279 following the early buzz surrounding a new theory (Koricheva et al. 2013).

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19 280 All analyses were performed using R v3.2.2 (R Development Core Team,
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21 281 2015). All code used in the analysis is included in the supplementary material.
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24 283 RESULTS

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27 284 Across all effect sizes there was no significant correlation between stress
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29 285 levels and the degree of secondary sexual signalling (intercept-only MCMCglmm;
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31 286 posterior mean = -0.08, LHPD = -0.22, UHPD = 0.03, $k = 118$, $N_{\text{studies}} = 38$, $N_{\text{species}} =$
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33 287 25). There is, therefore, no general signalling of level of stress by secondary sexual
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35 288 traits across species, stress measures, and traits. Total heterogeneity was large
36
37 289 however ($I^2_{\text{total}} = 77.81\%$). The amount of variance explained by the three random
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39 290 factors was small ($I^2_{\text{study}} = 26.76\%$, $I^2_{\text{species}} = 8.88\%$, $I^2_{\text{phylogeny}} = 5.59\%$), with
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41 291 substantial residual variance remaining after accounting for them ($I^2_{\text{residual}} = 36.59\%$).
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45 292 As there was substantial heterogeneity in the dataset, we next used a model-
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47 293 selection approach to investigate potential categorical moderators of effect size.
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49 294 Adding taxonomic class, sex, or stress measure as a categorical fixed-effect to the
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51 295 meta-analytic model did not improve the model fit (Table 1). Accordingly, none of
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53 296 the categories associated with taxonomic class, sex, or stress measure exhibited a
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56 297 mean effect size that was significantly different from zero (Table 2). However, model
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3 298 fit was significantly improved by the addition of secondary sexual trait type as a fixed
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5 299 effect (Table 1), suggesting this factor explains some of the observed heterogeneity in
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7 300 effect sizes. A meta-regression indicated that there is a significant negative mean
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9 301 effect size when considering only those effect sizes associated with opposite-sex
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11 302 preferences (Table 2), such that stress tended to be associated with mate preferences,
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13 303 with lower stress individuals favoured. However the the upper highest posterior
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15 304 density estimate is very close to zero (-0.010). There was no significant effect of the
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17 305 remaining three secondary sexual trait types.

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21 306 We found mixed evidence for publication bias in the dataset. Egger's
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23 307 regression suggested there was no significant funnel plot asymmetry ($F_{1, 116} = 0.1, P =$
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25 308 $0.75; \beta = 0.004, \text{intercept} = -0.03$). However, a trim-and-fill analysis on the residual
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27 309 effect sizes suggested that 13 effect sizes were "missing" from the right hand side of
28
29 310 the funnel plot. After imputing these missing effect sizes the mean effect shifted by
30
31 311 0.048 (Figure S1). Adjusting our original mean effect size estimate (from the intercept
32
33 312 only model) using this value still resulted in a non-significant result (mean = -0.032,
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35 313 LHPD = -0.169, UHPD = 0.075, $k = 131$). In terms of temporal patterns, we found no
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37 314 correlation between effect size and year (Spearman's rank correlation: $r_s = -0.03, P =$
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39 315 0.77). However all the studies included were published relatively recently (between
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41 316 2001-2014), and a temporal trend is probably unlikely over such a short range.

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46 47 318 DISCUSSION

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51 320 Our meta-analysis did not detect a significant relationship between
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53 321 physiological indices of stress and the expression of sexually-selected traits across 26
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55 322 vertebrate species from 4 taxonomic classes. Furthermore, we failed to detect effects
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3 323 of stress in any of the four vertebrate orders, in either sex, or when controlling for the
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5 324 measure of stress employed. However, we did detect a significant effect of the type of
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7 325 sexually-selected trait, such that stress had significant detrimental effect on opposite
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9 326 sex preferences, but not on the expression of colouration or vocalisation, or on trait
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11 327 size.

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14 328 Opposite sex preference is likely to reflect an aggregate response to the
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16 329 development of one or more sexually-selected signals and the true 'attractiveness' of
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18 330 an individual to the opposite sex (rather than relying on our judgements of the
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20 331 attractiveness of trait expression). While our results suggest that members of the
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22 332 opposite sex attend to cues of stress, we do not know which traits are used in their
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24 333 assessment. It is possible, for example, that there are behavioural traits in addition to
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26 334 the morphological traits that we have included here which provide cues to
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28 335 physiological status (Roberts et al. 2007). In addition, it is possible that effects of
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30 336 stress on colouration or vocalisation are more nuanced and complex than our analysis
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32 337 was able to detect.

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36 338 For instance, effects of stress on colouration may be dependent upon the
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38 339 nature of the colouration (e.g. melanic versus carotenoid), the context (e.g. breeding
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40 340 season versus moult) and species ecology (e.g. the mating system). Melanic
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42 341 coloration, for example, can provide insight into links between the stress response and
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44 342 sexual signalling as the melanocortins which control the expression of phaeomelanic
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46 343 coloration (Ducrest et al. 2008) also influence sensitivity to stressors (Ducrest et al.
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48 344 2008; Roulin and Ducrest 2011). There were, however, only a small number of
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50 345 studies in which melanic colouration was measured (8 effect sizes from 3 studies, see
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52 346 Table S3) meaning that it was not possible to test effects of stress on these separately.
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54 347 A greater number of studies measured carotenoid colouration ($n = 14$), and in a high
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3 348 proportion of those of avian species (21 of 27 effect sizes from 9 studies, see Table
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5 349 S3), stress had detrimental effects on carotenoid colouration. This colouration is
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7 350 dependent upon antioxidants acquired in the diet (McGraw 2006) which can be
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9 351 diverted away from secondary sexual traits and into reduction of oxidative damage
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11 352 under stress (Fitze et al. 2009). Although this may explain the pattern of stress-
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13 353 induced colour reduction in birds (but see Collins et al. 2008), there was some
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15 354 evidence that chronically elevated GCs enhanced ventral coloration in males of the
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17 355 common lizard (Cote et al. 2011). This discrepancy could stem from differences in
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19 356 species' ecology, meaning that it is adaptive for males of some species (e.g. the
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21 357 common lizard) to make a 'terminal investment' in mating under stress, perhaps due
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23 358 to reduced chances of survival (Pryke et al. 2007; Fitze et al. 2008; Bonier et al. 2009;
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25 359 Huyghe et al. 2009). In biparental mating systems, such as those of many bird species,
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27 360 the optimal solution to the allocation of energy under stress may be away from mating
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29 361 effort and into, for example, parental investment. It may not be possible, then, to
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31 362 detect any effects of stress on colouration until a sufficient number of studies across
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33 363 species and colouration type are available.

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38 364 We did not find an effect of stress on vocalisations. More than a third of these
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40 365 effect sizes were measured in anurans during the breeding season (14 of 37 effect
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42 366 sizes). A number of studies have shown glucocorticoids to be elevated across the
43
44 367 breeding season, with those individuals who vocalise the most showing the highest
45
46 368 levels (Gladbach et al. 2010; Goymann and Wingfield 2004). At threshold GC levels,
47
48 369 however, males change their strategy and stop calling, likely on reaching a negative
49
50 370 energy balance (Emerson 2001). As this threshold depends upon intrinsic (e.g.
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52 371 condition) and extrinsic (e.g. rainfall, chorus density) factors, despite the organising
53
54 372 role of GCs on calling strategy within individuals, consistent effects may not be easily
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3 373 detected across individuals and studies (Emerson 2001). The majority of the
4
5 374 remainder of effect sizes categorised as ‘vocalisations’ were for effects of stress on
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7 375 dimensions of birdsong. There is reason to predict that birdsong is linked to
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9 376 dimensions of the stress response as the brain centres responsible for song develop
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11 377 early in life, during which time conditions also determine adult stress resistance
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13 378 (Buchanan et al. 2004; Pfaff et al. 2007; Muller et al. 2010). Both may stem from a
14
15 379 common phenotype (Spencer and MacDougall-Shackleton 2011), rather than song
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17 380 responding to fluctuations in adult stress. Failure to find effects on vocalisation, then,
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19 381 may stem from the fact that relationships between stress and vocalisation are non-
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21 382 linear and context-dependent, and that effects on birdsong and amphibian vocalisation
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23 383 may be functionally different.
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27 384 Potential roles of stress on the expression of sexually selected traits have
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29 385 included indirect effects of glucocorticoids via the immune system (Møller 1995;
30
31 386 Buchanan 2000), body condition (Husak and Moore 2008), or testosterone (Buchanan
32
33 387 2000; Roberts et al. 2007; Husak and Moore 2008), or via sexual selection on the
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35 388 physiological stress response itself. In the former, the effects of stress on sexual traits
36
37 389 would likely be difficult to detect without measuring, and controlling for, its effects
38
39 390 on testosterone, immune function, and body condition. In the latter, sexual signals
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41 391 would provide cues to individual differences in dimensions of the stress response such
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43 392 as stress reactivity or the efficiency of negative feedback (Pfaff et al. 2007; Roberts et
44
45 393 al. 2007; Husak and Moore 2008; Bortolotti et al. 2009; Schmidt et al. 2012). While a
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47 394 number of studies included in our analyses reported various indices of immune
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49 395 function, testosterone, and body condition, there were insufficient numbers to test for
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51 396 their roles in our model. A promising avenue for future research is analysis of
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53 397 individual differences in the stress response, which may provide the necessary
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3 398 conditions for the evolution of condition dependent traits (e.g. Moore and Hopkins
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5 399 2009). Individual differences in stress reactivity and the efficiency of negative
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7 400 feedback, for example, are heritable (Rowe and Houle 1996; Evans et al. 2005; Korte
8
9 401 et al. 2005; Stowe et al. 2010), and production and effects of GCs are related to
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11 402 measures of genetic quality (Olsson et al. 2005) and fitness (Bonier et al. 2009). An
12
13 403 efficient stress response is likely to be comprised of low baseline GCs, moderate
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15 404 elevation, and rapid negative feedback once the stressor has passed (Olsson et al.
16
17 405 2005; de Kloet et al. 2008). While peak GC response to a standardized stressor was
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19 406 not significantly related to expression of secondary sexual traits in our analyses
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21 407 (although the sample size was small; $n = 9$ effect sizes, see Table S3), an inverse
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23 408 relationship between a sexual signal and sensitivity of negative feedback (Schmidt et
24
25 409 al. 2012) and a finding (excluded from our analyses due to lack of statistical
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27 410 information) that female zebra finches preferred males from lines bred for low peak
28
29 411 GC response (Roberts et al. 2007), further support this as an important future research
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31 412 direction.
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36 413 It is, however, extremely difficult to measure these dimensions of the stress
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38 414 response, particularly in free-living individuals. Regulation of glucocorticoids in
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40 415 response to predictable seasonal challenges such as the moult (Husak and Moore
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42 416 2008; Romero et al. 2005) or breeding (Kitaysky et al. 1999), for example, may have
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44 417 different effects on the allocation of resources to sexual signalling than those due to
45
46 418 unpredictable stressors (O'Reilly and Wingfield 2001). This demonstrates the need
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48 419 for multiple measures of the stress response, long-term stress, and stress history in
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50 420 future research. While this is undoubtedly difficult, records of local weather
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52 421 conditions, season, resource availability and population density, for example, could be
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54 422 controlled for in analyses. Repeated measures of stress provide a more ecologically
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3 423 valid assessment of the experience of stress (Bonier et al. 2009). More comprehensive
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5 424 measurements of the stress response, including duration, total GCs released in
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7 425 response to an ecologically valid, standardised stressor, and the efficiency of negative
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9 426 feedback (Romero 2004), provide a set of dimensions of the stress response with
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11 427 which to compare individuals. In addition, the concentration and distribution of GC
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13 428 receptors may be more meaningful measures of individual differences in stress
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15 429 reactivity in terms of effects on morphological and behavioural traits than GC
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17 430 production itself (Schmidt et al. 2012).

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21 431 Our analysis controlled for phylogenetic relatedness among species. The
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23 432 amount of variance in effect size explained by phylogeny was very small. This could
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25 433 be for several reasons. First, it may be that the relationship between stress and
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27 434 secondary sexual trait expression is highly evolutionarily labile, so that phylogenetic
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29 435 effects are important only for very closely related species. This may be especially
30
31 436 likely for those studies concerning male vocalisation, as behavioural traits such as
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33 437 these may evolve particularly rapidly (Blomberg et al. 2003), and is frequently seen in
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35 438 meta-analyses concerning behavioural traits (e.g Santos et al. 2011; Dougherty and
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37 439 Shuker, 2015). Alternatively, this could be an artefact of the fact that the average
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39 440 phylogenetic distance between species in our tree is relatively large (Björklund 1997).
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41 441 With such a tree the power to detect a phylogenetic signal is reduced, especially if
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43 442 there is substantial variation across species in factors (such as physiology or
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45 443 behaviour) that may affect the relationship we are investigating.

49 444 In conclusion, stress was not associated with the expression of sexually-
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51 445 selected traits in our sample. The results therefore challenge any notion of a common
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53 446 stress-signalling function for sexual signals. It was, however, associated with the
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55 447 strength of preference for the opposite sex, suggesting that stress is relevant to mating
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3 448 decisions but that our analysis was not able to detect the specific traits through which
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5 449 it is signalled. This means that stress may be important for mate choice, but it does not
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7 450 provide a simple explanation for the role of any one sexual display or signal. This
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9 451 discrepancy therefore needs resolving. Our findings add to the body of work which
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11 452 seeks to identify how stress can moderate the expression of physical and behavioural
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13 453 traits more generally (e.g. Lupien et al. 2009; Buchanan et al. 2013). We argue that in
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15 454 order to advance our understanding of roles of stress in sexual selection, we need to
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17 455 develop a model which incorporates the nuanced effects of species ecology, trait type,
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19 456 ecological context and the complex nature of the physiological stress response.
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3 862 FIGURE LEGENDS
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7 864 **Figure 1.** PRISMA flow chart showing results of literature search and study selection
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9 865 criteria and process. Table S1 shows studies excluded from analyses.
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13 867 **Figure 2.** Phylogeny included in meta-analysis. For details please main text.
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For Review Only

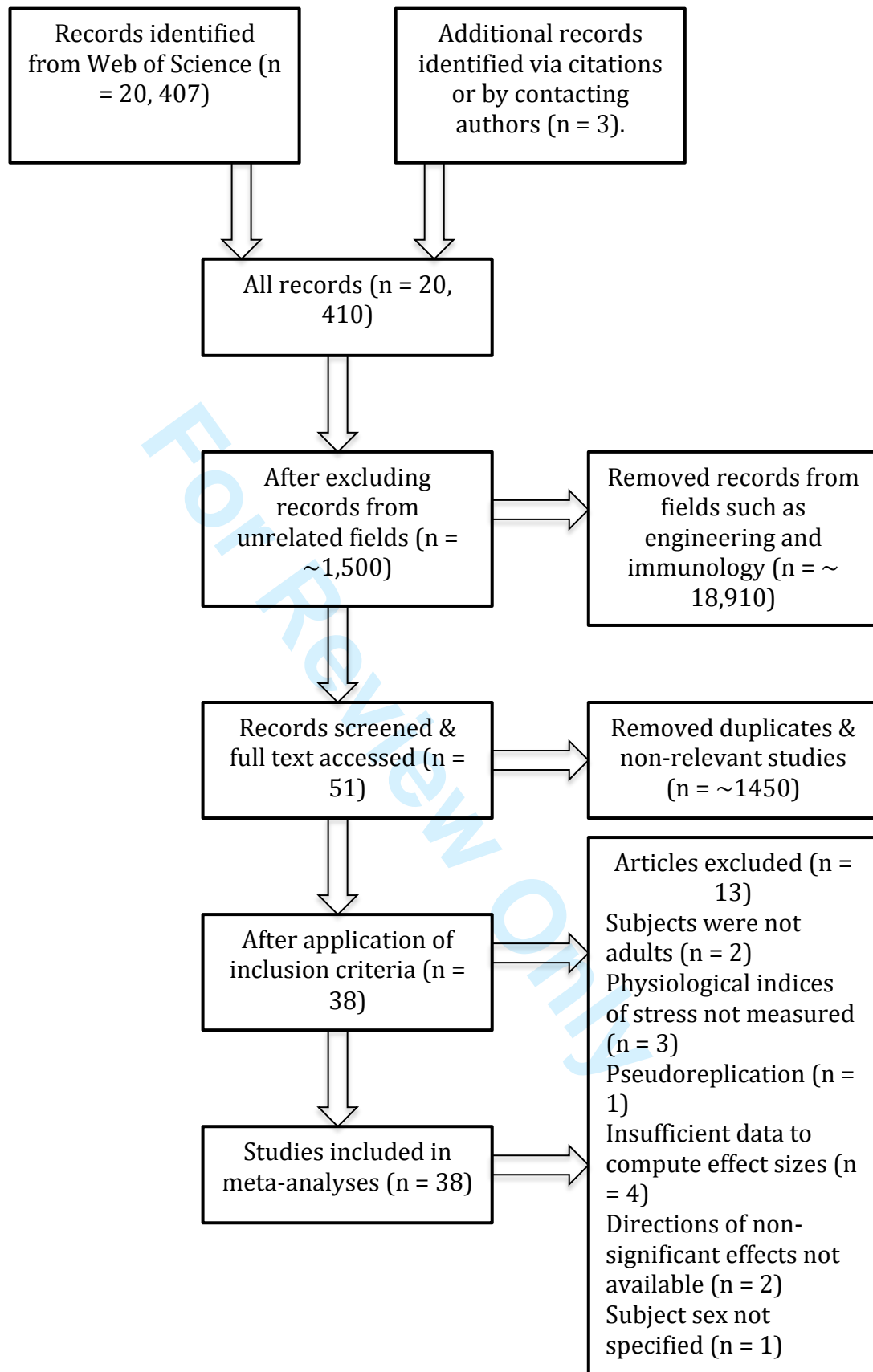
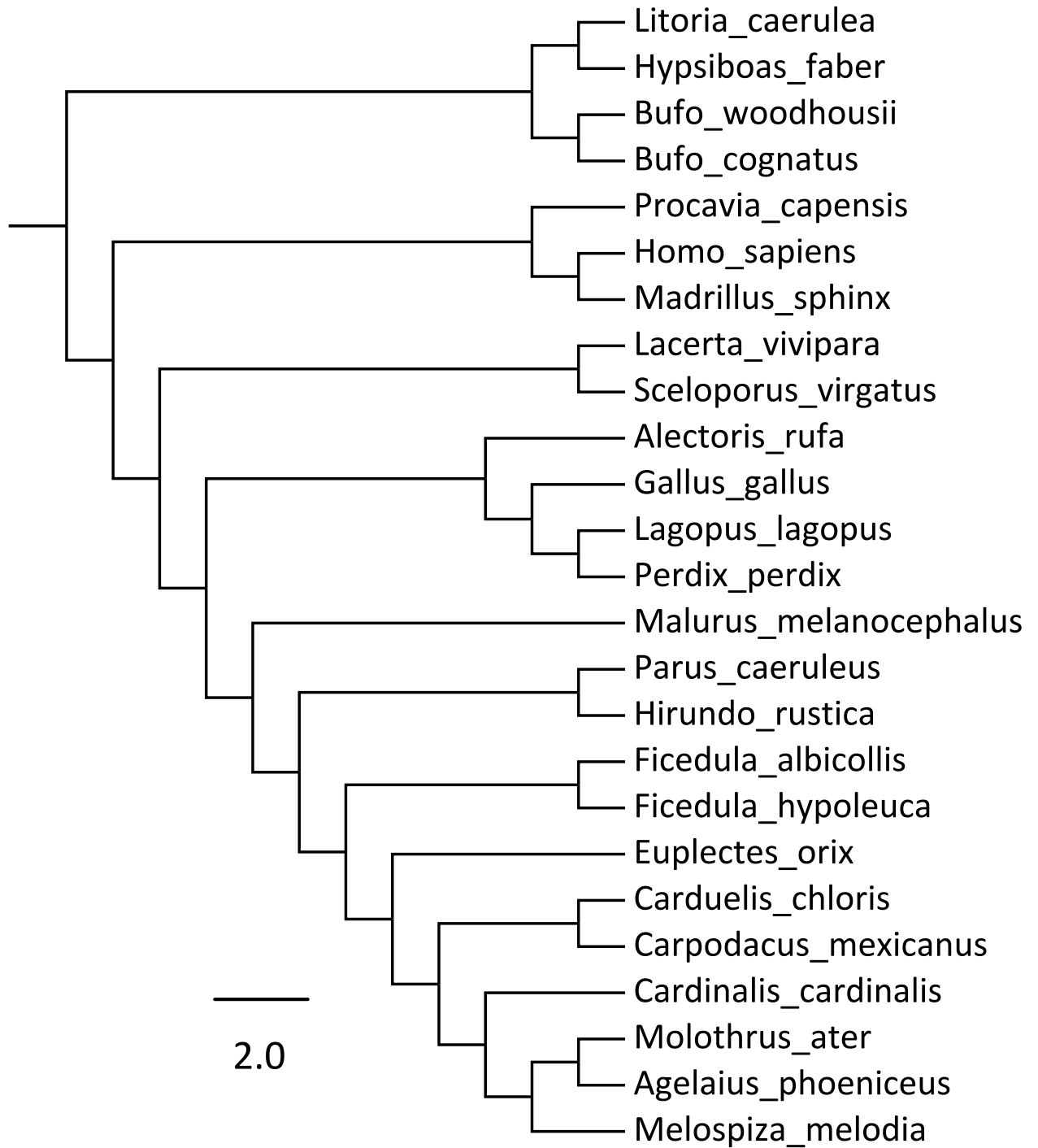


Figure 1. PRISMA flow diagram of search protocol and results and study selection.



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