Accurate systematic frameworks are vital to advance ecological and evolutionary studies, with an example from Australian freshwater fish (Hypseleotris)


AAustralian Rivers Institute, Nathan Campus, Griffith University, 170 Kessels Road, Nathan, Qld 4111, Australia.
BWater Planning Ecology, Department of Science, Information Technology and Innovation, 41 Boggo Road, Dutton Park, Qld 4102, Australia.
CEvolutionary Biology Unit, South Australian Museum, North Terrace, Adelaide, SA 5000, Australia.
DSchool of Biological Sciences, The University of Adelaide, North Terrace, Adelaide, SA 5005, Australia.
EFRC Environmental, PO Box 2363, Wellington Point, Qld 4160, Australia.
FMuseum & Art Gallery of the Northern Territory, GPO Box 4646, Darwin, NT 0801, Australia.
GInstitute for Applied Ecology, Building 3, University of Canberra, ACT 2601, Australia.
HCorresponding author. Email: penguintim@hotmail.com

Abstract. The practice of modern evolutionary and ecological research is interdisciplinary, with the process of evolution underpinning the diversity on display. However, the inference of evolutionary patterns can be difficult owing to their historical nature. When the biological units and evolutionary relationships involved are unclear, interpreting any ecological and biological data can be problematic. Herein we explore resulting issues when evolutionary theories rely on an unclear or incomplete biological framework, using some Australian freshwater fish (carp gudgeons: Hypseleotris, Eleotridae) as an example. Specifically, recent theories regarding the role of developmental plasticity on ontogeny and speciation have focused on this group. However, carp gudgeons have complex, and as yet incompletely understood, species boundaries and reproductive biology. Even basic data for the recognised taxa, relating to their phylogenetic relationships, life histories and species distributions, are unclear, have often been misinterpreted and are still in the process of being assembled. Combined, these factors make carp gudgeons a relatively poor group on which to apply more advanced evolutionary theories at the moment, such as the role of developmental plasticity in diversification.

Additional keywords: carp gudgeons, developmental plasticity, Eleotridae, life history, phylogenetics, species distributions, taxonomy.

Introduction
The modern study of biology incorporates a huge range of subdisciplines, many of which seem fairly obscure to non-practitioners or involve highly specialised methods, and yet all are neatly tied together by the underlying process of evolution. This is an idea most eloquently expressed in Theodosius Dobzhansky’s (1973) essay ‘Nothing in Biology Makes Sense Except in the Light of Evolution’. Although evolution is the underlying narrative that explains much of the biodiversity and ecological processes that we see around us, it is particularly difficult to study owing to its historical nature. We only have one replicate to consider, that of life on Earth, so true experiments are difficult to run and involve trying to infer scenarios of what has already happened (Mayr 2000). This retrospective view can lead to problems of interpretation. Evolutionary patterns result from a large number of often unknown processes. This means that we need a strong framework of knowledge to make sense of the patterns that confront us. This scientific framework is constructed from a combination of theories and real data. If either of these is not robust, then our understanding of evolution and ecology will be hampered.

The evolutionary patterns that form the basis for both developing and testing theories need to be clear and reasonably well understood within a proper biological systematic context before they can be properly used. By context, we mean precisely which species are being studied? And when considering the
evolutionary origins of that species and its characters, then exactly how do these species relate to other taxa? Without a clear delineation of these units and relationships, any conclusions on the evolution of associated biological and ecological factors, such as behaviour or biogeography, may be spurious, out of context and largely orphaned from sound evolutionary theory.

Recently, a theory of the role of developmental plasticity on ontogeny and its effects on the generation of diversity of species and life history traits has been put forward as a possible and underappreciated driver of biodiversity (Vilizzi and Kováč 2014). The basic idea is there are several different biological ways (life history ‘canals’) in which an individual (and then population) can develop, depending on the situation in which it finds itself (Waddington 1942), leading to several distinctly different adaptive optima (Pennig et al. 2010). One way these changes can be wrought is through epigenetic effects, where the external environment may influence the expression and regulation of genes as an individual develops during its lifetime (ontogeny), leading to distinctly different phenotypes and life histories (Verhoeven et al. 2016), and perhaps eventually to distinct species (Pennig et al. 2010). Vilizzi and Kováč (2014) put forward a framework of ‘alternative ontogenies and developmental plasticity’ (AODP) as a way of understanding biological diversity in a seeming contrast to more incremental, evolutionary development (with its strict genetic controls in combination with selection). Vilizzi and Kováč (2014) see the life histories of species and populations as potentially plastic, changing through time on a continuum from generalised (termed altricial) to specialised (precocial) and back (Balon 2004), and believe this process may be responsible for the diversity seen within species complexes.

As a case study, Vilizzi and Kováč (2014) applied their alternative framework to a group of fish from eastern Australia, namely the carp gudgeons (Hypseleotris, Eleotridae), where there are at least six species: three formally described and three informally described. This group comprises an unresolved complex of sexual and hybridigenetic taxa (Bertozzi et al. 2000; Schmidt et al. 2011), with varying life histories and distributions. Vilizzi and Kováč (2014) explain the evolutionary patterns seen in several of these taxa in terms of presumed epigenetic changes due to the complex interactions between genotype and phenotype in the face of the local environment. In particular, they suggest their framework can explain: (1) the different life histories of a pair of sympatric species in coastal eastern Australia (firetail gudgeon Hypseleotris galii and empire gudgeon Hypseleotris compressa); (2) the presence of two allopatric forms within the western carp gudgeon (Hypseleotris klunzingeri) in coastal and inland areas; and (3) another sympatric species ‘pair’ of carp gudgeons in inland eastern Australia (Midgley’s Hypseleotris sp. 1 and Murray–Darling Hypseleotris sp. 3; Ummack 2000; Allen et al. 2002).

However, at this stage carp gudgeons are a particularly problematic group to use as a test system from which to draw evolutionary and ecological inferences. This genus has been described as a ‘taxonomic trap’ (Hammer et al. 2013) owing to its numerous sexual species, many of which are only in the process of being delineated (P. J. Ummack and M. Adams, unpubl. data). The Hypseleotris of eastern Australia also include multiple hybridigenetic lineages that practise sexual parasitism (Lehtonen et al. 2013), some of which comprise male-only and female-only populations (Schmidt et al. 2011) and even retain evidence in their genes of a species not yet found in its pure sexual form (HX; sensu Bertozzi et al. 2000).

Leaving aside the vexed and developing linked issues of species boundaries and reproductive biology within Australian carp gudgeons, we explore the suitability of this group to serve as the raw material for more derived evolutionary theories using even the currently recognised six species in eastern Australia. In particular, we consider complexities and pitfalls of interpretation of their detailed phylogenetic, life history and distributional patterns. Understanding a process (such as developmental plasticity) requires the accurate identification of patterns, but the species ‘complexes’ within these fish truly lives up to the name, with relevant biological units still very unclear. This makes more advanced ecological and evolutionary inferences, such as developmental plasticity on ontogeny, very difficult and potentially premature.

Interpreting phylogenetic patterns

A framework of evolutionary relationships (phylogeny) provides the opportunity to relate phylogenetic history with life history, as well as other species traits, in an attempt to understand evolutionary trajectories. However, any such interpretations derived from a phylogeny are only as strong as that phylogeny. Vilizzi and Kováč (2014) use Australian carp gudgeons as the example of their framework of alternative ontogenies and developmental plasticity. The carp gudgeon phylogeny of Thacker and Ummack (2005) provides the evolutionary patterns, which is thus a key element of the argument of Vilizzi and Kováč (2014). This topology recovers western carp gudgeon as the sister taxon to the remaining Australian carp gudgeons (including the empire and firetail gudgeons), leading Vilizzi and Kováč (2014) to class the western carp gudgeon as a ‘basal species’ (‘first branching lineage’ is a more appropriate phrase). However, this phylogeny is based on only a single mitochondrial gene (NADH dehydrogenase subunit 2 (ND2)). Although the phylogeny also includes 20 morphological characters, these are insufficient to differentiate the number of terminal taxa included, and are swamped by the molecular characters, which leads to the same topology when the morphological characters are not included. Further, the clade of the remaining Australian species, which causes the western carp gudgeon to become the ‘basal’ lineage, is only very weakly supported (lowest possible support of decay index = 1). Another more recent topology of carp gudgeons (Thacker et al. 2007) does not recover western carp gudgeon as sister to the other species, and is itself only based on a single mitochondrial gene (cytochrome b (CytB)).

We explored the phylogeny of this group further by performing some preliminary analyses of our own using various new data now available on GenBank (www.ncbi.nlm.nih.gov, accessed 13 June 2016) as an example of more preferable multilocus analyses. Because of the many issues of unclear biological units among Hypseleotris, any results need to be approached cautiously, in particular any species labels applied to Midgley’s, Murray–Darling and Lake’s carp gudgeons because of uncertain identifications. We downloaded sequences
Thacker and Unmack (2005) is accurate, it may be a reasonable and potentially wrong framework for carp gudgeons has not yet been fully developed. One nuclear locus (18S rRNA) observed in eastern Australia (P. J. Unmack and M. Adams, unpubl. data), thus implying a long period of reproductive isolation. So, if we posit that the western carp gudgeon is the ‘basal’ lineage, that brings us to another caveat in using phylogenies to interpret character evolution: ‘basal’ does not mean ‘ancient’ or ‘primitive’. Early branching taxa in a phylogeny of extant species are often described as ‘basal species’, but this is not accurate. A species is not basal as such; rather, the node that leads to it may be (Crisp and Cook 2005) and either side of a tree could equally be termed ‘basal’ (Krell and Cranston 2004). The only thing implied by a ‘basal lineage’ is that it is a sister to the remaining taxa in the tree. It is not older, because by definition sister taxa are of exactly equal age. Often when there is a single early branching lineage that contains only one extant species, it is then thought to somehow be older or more primitive, as assumed by Vilizzi and Kováč (2014). However, that lineage may not contain any other species merely because of some recent extinctions or poor sampling. There has been precisely as much time for natural selection and other factors to have acted upon a ‘basal’ western carp gudgeon lineage as for the putative other lineage containing the remaining Australian carp gudgeons.

Because ‘basal’ species have often been assumed to be older, they are frequently considered to be more like the ancient, ancestral form (pleisiomorphic; Crisp and Cook 2005). Vilizzi and Kováč (2014) suggest that the western carp gudgeon is ‘the basal form’ and then say that one of the coastal species ‘pair’, namely the empire gudgeon, evolved into its more generalist (‘altricial’) lifestyle whereas the other (firetail gudgeon) evolved into its more specialised (‘precocial’) lifestyle from the basal form. This is of course possible, but the phylogeny

![Fig. 1. Inset. Bayesian phylogenetic hypothesis for combined dataset of five loci (18S, 12S, cytochrome c oxidase subunit I (COI), cytochrome b (CyB) and NADH dehydrogenase subunit I (ND2)) from eastern Australian Hypseleotris species, with representatives of other eleotrid genera. Sub., substitutions. Posterior probability support values of nodes with >0.50 are shown. The tree score is −16 996.20. The graph shows principal coordinates analysis (PCoA) of 14 morphological, ecological and life history traits for 33 species of Australian freshwater eleotrids (data extracted from Sternberg and Kennard 2014), with trait vectors scaled by correlation coefficient (continuous variables are vectors; nominal or ordinal variables are points in ordination space). Four variables had a significance level >0.1 so were excluded (shape factor, swim factor, vertical position benthic and spawning frequency). The first two axes explain 68.8% of the total variation in species traits. Carp gudgeons (Hypseleotris) are indicated by triangles and labelled by species; others are indicated by circles and are labelled by genus only. The fish drawing (Pusey et al. 2004) shows Hypseleotris compressa (by Brad Pusey, used with permission).](image)
cannot be used as evidence for this because of an uncertain or contradictory topology, and because of a misinterpretation of the idea of basal taxa.

**Interpreting life history patterns**

Interpreting phylogenetic patterns plainly has its pitfalls, which can directly affect the interpretation of life histories, which have their own difficult issues. Vilizzi and Kováč (2014) point out that the empire gudgeon has ‘ancestral’ reproductive characters (such as small eggs), even though they do not seem to consider it as an ancestral form. They do not mention that the likely sister to all the Australian carp gudgeons is the very widespread Indo-Pacific species *Hypseleotris cyprinoides* (Thacker and Unmack 2005), which is similar to the empire gudgeon, with tolerance for salt water and an amphidromous-like life history (Thacker and Hardman 2005). To follow the logic of Vilizzi and Kováč (2014) on early branching lineages, this lifestyle should then represent the ancestral type, making the empire gudgeon more akin to the ancestor and the western carp gudgeon more derived.

Because there is unequivocal evidence for reproductive isolation and significant genotypic divergence between various combinations of these extant sympatric taxa (Bertozzi et al. 2000; Schmidt et al. 2011), Vilizzi and Kováč (2014) appear be addressing the role of life history plasticity in the ancient speciation processes that led to these taxa, rather than suggesting any current plasticity, which would have implied its subsequent loss in the contemporary daughter lineages (Pfennig et al. 2010). Phylogenetic studies can lead to an inference of evolutionary patterns resulting from speciation and an attempt to understand the mechanics of the processes, but precisely what happened or patterns resulting from speciation and an attempt to understand the mechanics of the processes, but precisely what happened or why things developed as they did will always be difficult to know at such a distance (Moczek 2015). Further, as far as we are aware, there is no evidence of this type of life history plasticity in contemporary populations of carp gudgeons; even if there were, that would not mean that the ancestors were similarly plastic.

Contemporary carp gudgeon life histories are a key factor in the arguments of Vilizzi and Kováč (2014), but they lack clarity because the data used were limited. As with the phylogenetic analyses above, we explored these life history patterns further by incorporating more detailed data, and these also come with the proviso that any of the ‘species’ life history data could be confounded by the unrealised presence of incorrect identifications, cryptic species and hybrids, and are presented more by way of an example. We considered a suite of 14 morphological, ecological and life history traits (data from Sternberg and Kennard 2014; for traits, see Fig. 1) from all the Australian carp gudgeon taxa mentioned by Vilizzi and Kováč (2014), as well as other Australian freshwater species from the gudgeon family (Eleotridae), for a total of 33 species from 10 genera. We summarised and compared interspecific differences with principal coordinates analysis (PCoA) in R, ver. 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria, see www.R-project.org), using a species-by-trait dissimilarity matrix and Gower’s coefficient. Traits with the highest contribution to interspecific variation were fit to the plot with ‘envfit’ in the Vegan package, where vector lengths are scaled by their correlation coefficient. In line with our expectations, the *Hypseleotris* species grouped together closely with the exception of the empire gudgeon (*H. compressa*), which was consistently distinct and much closer to other genera (e.g. *Eleotris*, *Giurus*, *Gobionotus*), which share its salinity tolerance and general amphidromous-like lifestyle (Fig. 1), despite it clearly belonging within *Hypseleotris* phylogenetically (Fig. 1, inset). The sympatric inland species ‘pair’ (sensa Vilizzi and Kováč 2014), namely Midgley’s and Murray–Darling carp gudgeons, are nearly identical for these traits and do not show any obvious split, although the issue of accurate delineation of biological units may make this and other *Hypseleotris* life history summaries suspect, calling into question any theories based on them.

The role of past plasticity in generating contemporary life histories is likely to remain unclear, but the scenario of a western carp gudgeon-like ancestor giving rise to groups of species pairs at different positions on a life history spectrum that can thus coexist is not supported by the best existing data.

**Interpreting distributional patterns**

As well as phylogeny and life history, simple spatial distribution of the taxa of interest can be very important data in disentangling and understanding evolutionary and ecological patterns. For example, the current distribution of a species can provide us with a great deal of ecological information, such as the particular habitat it prefers, while also giving strong hints as to its evolutionary and biogeographic history, because the species will have needed the opportunity and ability to arrive at its location at some time in the past. Whether taxa are allopatric (naturally found in different locations) or sympatric (taxa of interest found in the same location) has been an important factor in biological research (Hammer et al. 2013). For example, the speciation of allopatric sister species separated by an obvious geographic barrier is frequently considered to have been driven by the local geography (Unmack 2013). Similarly, sympathy can be an important factor that aids evolutionary understanding, because the existence of non-interbreeding populations in the face of sympathy is evidence of reproductive isolation and therefore full biological species status (Hammer et al. 2013). Sympathy is also important in theories of niche partitioning and character reinforcement (Noor 1999), where populations of different taxa may develop more pronouncedly different ecological and morphological traits when confronted with each other so as to avoid interbreeding or competition, a process in which plasticity itself could play a role (Pfennig et al. 2010). Indeed, spatial distribution patterns feature prominently in the example framework adopted by Vilizzi and Kováč (2014) to explain species complexes, namely the sympathy of two gudgeon species (empire and firetail) with distinctly different life histories in a ‘stable’ environment, the ‘predominant’ distribution of western carp gudgeons inland and the allopatry of two western carp gudgeon lineages.

However, species distributions only appear to be simple. The distribution of any taxon is a complex and changing interaction of many factors, including environment, habitat connectivity, life history and community interactions, among many other factors (Sternberg and Kennard 2013). These factors may be truly contemporary, hangovers from the distant past or both. Each taxon finds its own way to its current distribution (Page and Hughes 2014) and may or may not have been significantly
influenced by the presence of other species or the current environment; thus, inferring precise explanations for distributions (as with past life history patterns) is extremely difficult. This is particularly so given the extent of anthropogenic changes recently wrought on the entire landscape, including extinctions, introductions, water resource development and land degradation (Davies et al. 2010). Further, there is no reason to suppose that any taxon is at equilibrium (MacArthur and Wilson 1967) and may not have yet achieved its ‘final’ distribution, in which case trying to then parse the data from a contemporary distribution to draw out any explanatory factors will likely not tell a complete story.

Even if we were to assume that equilibrium has been reached, there are still numerous caveats in interpreting contemporary distributions. For example, when species are frequently difficult to correctly identify, as are carp gudgeons, this may distort any distributional information (as with phylogenetic and life history data), as appears to be the case for firetail and Midgley’s gudgeons (Pusey et al. 2004). Further, the difficulties inherent in the presence of numerous known but undescribed carp gudgeon species (Unmack 2000), ‘cryptic’ species and numerous, site-specific combinations of sympatric sexual congeners plus hemiclinal lineages, many potentially capable of interbreeding, makes a definitive distribution of any carp gudgeon taxon highly suspect. This was the case in a recent fish survey, in which various combinations of sexual and hybridogenetic and native and introduced carp gudgeons could only be identified with molecular techniques (Hammet et al. 2012).

But even in an idealised world where we would know precisely what everything is and exactly where it is now, what does sympatry really mean? Does every site need to have all the relevant taxa, or just some? How many need to be shared to achieve ‘sympatry’? What about parapatric distributions, where taxa are adjacent or occasionally sympatric at the edges of their distributions? Vilizzi and Kovač’s (2014) main example is the sympathy of empire and firetail gudgeons in coastal eastern Australia, despite their different life history strategies. Vilizzi and Kovač (2014) infer that these sympatric species became both more and less specialised from a western carp gudgeon-like ancestor due to developmental plasticity. Leaving aside the systematic and life history questions raised above, are these species really sympatric? The empire gudgeon is a wide-ranging, mostly tropical, salt-tolerant species that is found along a coastal strip covering most of the Australian continent and ranging, mostly tropical, salt-tolerant species that is found along a coastal strip covering most of the Australian continent and is an obligate freshwater species, restricted to subtropical to temperate climates, with a narrow coastal distribution from central Queensland to central New South Wales (Unmack 2000). It is not confined to lowlands, generally being within 150 km of river mouths (Pusey et al. 2004). So, although both species can be found together, for the overwhelming proportion of empire gudgeon’s distribution there are no firetail gudgeon present. But even if we look at the river systems where both species are resident, the firetail gudgeon is more likely to be found further upstream. Rose et al. (2016) present data from a region where the distributions of the two species overlap. There were 118 sites where one or both species were present, finding both species sympatric at 44% sites, with 48% of sites only hosting firetail gudgeon (at more upstream sites). The relatively thin strip of particular lowland habitat occupied by empire gudgeon may imply that it is this species that is the true specialist. However, more detailed surveys that include a total evidence approach would make the situation clearer.

Vilizzi and Kovač (2014) concentrate on the sympathy of empire and firetail gudgeons in parts of eastern Australia, but there are many other cases of sympathy within carp gudgeons. Midgley’s carp gudgeon is also commonly caught together with firetail gudgeon (when they have been accurately differentiated), and firetail gudgeon is sympatric with western carp gudgeon at 56% of sites sampled in south-eastern Queensland (Pusey et al. 2004). Multiple different forms of carp gudgeon have been reported from the Burdekin River (Pusey et al. 2004), and this does not even begin to cover the similarly complex situation for various combinations of taxa found throughout the inland Murray–Darling system (Hammer et al. 2012) or Cooper Creek in the Lake Eyre Basin. This all calls into question the relevance and potential evolutionary influence of the current partial sympathy of one particular pair of fish species in one area.

Another case study of Vilizzi and Kovač (2014) involves the allopatric twin ‘forms’ of western carp gudgeon, one from the more ‘variable’ environment of inland Australia and the other from the more ‘stable’ east coast. Vilizzi and Kovač (2014) suggest that possible morphological differences between them may also represent the same process of specialisation that they had inferred for the empire–firetail species ‘pair’, due to developmental plasticity. Leaving aside the probability that these western carp gudgeon ‘forms’ represent different biological species (Thacker et al. 2007), that any morphological differences between inland and coastal populations are still very unclear and that many short coastal rivers of eastern Australia are not particularly stable (Kennard et al. 2010), this pattern sounds very like standard allopatric divergence and incipient or full speciation.

Importantly, the above examples of sympathy also highlight another caveat when interpreting distributional data. Despite discussing contemporary distributions, Vilizzi and Kovač (2014) are really invoking past distributions, because the speciation and divergence events that led to the current diversity of life histories on display are relatively ancient. The implications seem to be that ancestral western carp gudgeon must have been in the ‘variable’ inland environment when they evolved their current life history patterns, and that ancestral firetail gudgeon and more derived coastal western carp gudgeon must have been in ‘stable’ coastal environments when they did the same. Exactly how Midgley’s carp gudgeon (with a similar life history to the firetail, but found both inland and on the coast; Pusey et al. 2004) or Murray–Darling carp gudgeon (similar life history to the firetail gudgeon, but found inland) fit the alternative framework of Vilizzi and Kovač (2014) is ambiguous.

Clearly, if there are demonstrated uncertainties about current species distributions, then historic distributions remain largely opaque to us. Western carp gudgeon are found both on the coast...
and inland, and we do not know from which area they may have originated (perhaps neither); similarly, the ability to interpret many species’ geographic origins is challenging (Unmack 2013). Individuals plainly move, or the various species would not have large distributions, but in exactly what order and when they did so is difficult to know. As for the mapping of traits to evolutionary history, it is possible to infer ancestral areas (as well as life histories) for taxa in a phylogeny, but the answers derived are variable and frequently inaccurate (Crisp and Cook 2005).

Ironically, it may not really matter too much whether these various taxa are sympatric now or in the past. It may be that inter- and intraspecific competition is a relatively weak structuring force when it comes to fish assemblage composition, because interactions between species are likely mediated by habitat (Arthington et al. 2005). This is highlighted by the apparent success of invasive species in new environments. As long as reproduction, food and habitat conditions are met, new arrivals often flourish regardless of the species already present (Moyle and Light 1996). The effect of sympathy (through competition, selection etc.) may be relatively small and weak compared with the effect of habitat (and other species) for populations that are, or were, allopatric (Noor 1999). Short-term variability in environmental conditions will drive optimisations in traits that are more labile, whereas traits that are constrained by phylogeny will be more responsive to long-term variation in environmental conditions (Kellermann et al. 2012).

Again, this suggests that habitat template may be a better predictor of assemblage structure than competition.

This has been demonstrated to some extent for the carp gudgeons. Although the simple distributional data mentioned above obviously have their limitations, when combined with various methods of species distribution modelling, explanatory variables can be extracted and predictions made. Much of this work, which has included various carp gudgeon species, as well as many others, has indeed shown the prevalence of environmental, hydrological and habitat variables (as filters) in explaining both the distribution of particular species (Kennard et al. 2007) and the distribution of life history traits (Sternberg and Kennard 2013; Sternberg et al. 2014). Stewart-Koster et al. (2013) specifically consider firetail gudgeon and show the importance of both large-scale landscape and local-scale hydrological and habitat factors in understanding its realised distribution, which tends to be in the mid- to upper catchment. Conversely, the accuracy of the model-based prediction of the presence of empire gudgeon in Rose et al. (2016) is very high (95%), likely because of its specialised lowland habitat preferences. But does this mean that one can ignore biology and only consider the environment in understanding distributions? No, because there is a correlation between environmental variables and functional traits (Sternberg and Kennard 2013) and phylogeny is an important component in trait distribution (Sternberg et al. 2014). This is obviously intuitive, because the very reason that a particular habitat may be conducive to a particular fish species probably relates to that species’ traits. So, a powerful way to research and use distributional data is in the light of a strong framework of trait, habitat and phylogenetic variables, making predictions more flexible, accurate and informative (Rolls and Sternberg 2015).

Discussion and conclusion

Because of the demonstrated uncertainties prevalent among Australian carp gudgeons, which relate to their evolutionary relationships, species boundaries, life histories, distributions and basic reproductive biology, a basic framework of understanding on which to hang a theory like developmental plasticity is clearly not yet present. Plasticity may well be a factor in the past speciation of many species, but the theory behind it is itself still being developed and the evidence being collected (Pfenning et al. 2010). So, it may be possible that the idea of alternative ontogenies and developmental plasticity (Vilizzi and Kováč 2014) has played a role in the distribution of life histories and species within the Australian carp gudgeons, with past plasticity leading to eventual, or even rapid, speciation (Pfenning et al. 2010), be it allopatric or sympatric, and in the face of selection or drift or both. But the evidence does not yet show this, especially given the many unknowns and complications associated with this group.

The inference of past developmental plasticity would ideally be achieved with large comparative diversification analyses (Pfenning et al. 2010) or the good luck of having an extant ‘ancestor’ (Cresko et al. 2007). Developmental plasticity is most obvious and most easily studied at the intraspecific level, and so contemporary individuals and populations within a species (with a clear affiliation to delineated biological units) need to be studied in detail with whole-genome and epigenome scans (Verhoeven et al. 2016) and then related to RNA expression through transcriptome analysis (Wang et al. 2009). In the absence of these more formal analyses, it would be premature to interpret the many unclear patterns within carp gudgeons as the result of ontogenetic and developmental plasticity. This is especially so because the infinitely adaptable idea of plasticity could be invoked to explain almost any pattern, summed up by ‘epigenetics is a useful word if you don’t know what’s going on – if you do, you use something else’ (A. Bird, quoted in Verhoeven et al. 2016).

If ontogenetic and developmental plasticity is not the current best theory to explain the diversity of Australian carp gudgeons, is there a better one? A fairly basic historical biogeographic explanation of the diversity of carp gudgeons through allopatric divergence fits the patterns just as well as any based on unknown past plasticity. There are demonstrated influences of geographic barriers on the evolution of diversity in freshwater fish (Unmack 2013), including for most of these species in eastern Australia (Page and Hughes 2014). Amphidromy may well be an ancestral way of life for the whole family (Thacker and Hardman 2005). This interpretation makes sense biogeographically, with a highly vagile amphidromous ancestor colonising Australia, with one descendent lineage (empire gudgeon) retaining a similar life style and the rest adapting and evolving to a more sedentary freshwater life as required for colonising large continental rivers. Therefore, the Australian carp gudgeons likely represent a common case of differential adaptation of species to freshwater habitats from a more marine or estuarine inclined ancestor (Sternberg and Kennard 2014), as has also been inferred for freshwater gobies (Yamasaki et al. 2015), shrimps (Page et al. 2005; Cook et al. 2006) and prawns (Ashelby et al. 2012). These biogeographic and life history inferences for carp gudgeons
were only made possible after a systematic framework was developed, although they are only preliminary in the face of the many uncertainties within this group. However, ongoing research is strengthening our framework of understanding (Schmidt 2015; P. J. Unmack and M. Adams, unpubl. data).

We agree with Vilizzi and Kovič (2014) on the need for more integrated studies in ecology and evolution relating to these particular fish and to all species. In fact, we think that this broader holistic perspective has already begun and is accelerating, with the integration of multiple techniques evident within work on Australasian freshwater fish. The life history, reproductive biology, population biology and feeding ecology of various species have been explored with a combination of different genetic techniques and otolith microchemistry (Woods et al. 2010), stable isotopes (Cook et al. 2007; Villamarin et al. 2016) and intraspecific trait distribution (D. Sternberg, D. J. Schmidt, M. J. Kennard, S. R. Balcombe, N. A. Rickard and S. E. Bunn, unpubl. data). As suggested by Vilizzi and Kovič (2014), rainbowfish (Melanotaenia) have become a focus of research on potential plasticity, both for gross morphology (Lostrom et al. 2015) and the molecular pathways that underpin variation (McCairns et al. 2016). Further, genetics has been integrated into taxonomy (Larson et al. 2013), larval and gut contents identification (Hardy et al. 2011), food web studies (Hardy et al. 2010) and trait evolution (Mc Dowall and Waters 2004).

We welcome the full integration of developmental plasticity and ontogeny with genetic and other data into evolutionary and ecological research, which we see as complementary rather than an alternative to current and future work. As we point out above, there are numerous caveats when approaching evolutionary research, but when these are appreciated and fully accounted for, considerable advances will be possible in evolutionary and ecological understanding. We can only proceed further with a firm knowledge of the basic evolutionary units involved and their relationships, and so the ongoing work to disentangle the wildly interesting reproductive biology of these fish (Schmidt et al. 2011; Schmidt et al. 2013) is a necessary precondition to much further work. We think that a strong understanding of evolutionary frameworks will help set the stage for future ecological and evolutionary studies of all descriptions.

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