LETTER

Reply to Braasch and Postlethwait: Evolutionary origin of the teleost A2 agouti genes (*agouti signaling protein 2* and *agouti-related protein 2*) remains unclear

Tetrapods express one agouti gene [*agouti signaling protein* (*asip*)] in the skin regulating pigmentation and a second [*agouti-related protein* (*agrp*)] in the brain regulating energy homeostasis. In contrast, most teleosts have four genes encoding agouti-type proteins. Braasch and Postlethwait (1) present an analysis of the evolutionary history of agouti genes in teleosts, reordering the phylogenetic tree proposed previously (2) and proposing a nomenclature change. However, gene nomenclature derives mainly from functional or sequence properties and not from evolutionary predictions. Furthermore, inclusion of an ancient agouti sequence from the elephant shark provides an additional interpretation of the phylogeny (Fig. 1).

The word agouti, from the Guaraní language of South America, refers to rodents (genus: *Dasyprocta*) noted for prominent-banded pigment patterns in individual hair shafts. The term agouti came to be used to describe mutations in tetrapods that impacted this banding pattern. The *agrp* gene, discovered later, encodes a protein in hypothalamic neurons, and zebrafish *agrp* shares the same distribution of expression, regulatory properties, and function with the mammalian protein of the same name. More recently, we showed that a zebrafish gene called *agrp2*, a teleost-specific agouti family member expressed in the pineal, controls expression of hypothalamic melanin-concentrating hormone genes (*pmch* and *pmchl*) and regulates background adaptation (3).

In teleosts, an ancient duplication produced four agouti genes. *asip2* and *agrp2* (4) were initially called A2 genes, and their cysteine motifs start with C6C5CC… rather than C6C6CC… found in both *agrp* and *asip*. The analysis by phylogeny and synteny of teleost agouti genes (2) suggested that *agrp* and *agrp2* shared synteny with mammalian *agrp* and were paralogous genes derived from the teleost genome duplication. After the analysis of Braasch and Postlethwait (1), it seems unlikely that *agrp2* came from *agrp* and *asip2* came from *asip*, and it seems that the A2 genes could have a common origin. Why did Kurokawa et al. (2) suggest the original nomenclature? It seems that synteny analysis by Kurokawa et al. (2) was not based on the current scaffolds for *Takifugu rubripes* but an older version that has changed as the scaffolds improved.

We (H.B.S. and Å.V.) performed a phylogenetic analysis using Braasch and Postlethwait's (1) sequence set and included the ancient elephant shark *agrp*/*asip*-like sequence (5). This analysis suggested that *agrp2*s and *asip2*s are similar to each other (bootstrap value of 52–59% using maximum likelihood), in agreement with Braasch and Postlethwait (1). However, it is unclear from the phylogeny if the A2 cluster originated from the *asip* or *agrp* clusters. When the *agrp* cluster is used to root the tree (Fig. 1*A*), the A2 cluster groups with the *asip* cluster, but when the elephant shark *asip* is used to root the tree (Fig. 1*B*), the A2 cluster groups with the *agrp* cluster. It is, thus, doubtful that phylogenetic analysis of current sequences can determine if the A2 cluster is more similar to either *asip* or *agrp* clusters. In the meantime, according to the zebrafish nomenclature guidelines, the name that appears first in the literature should be given priority (http://zfin.org/zf_info/nomen.html).

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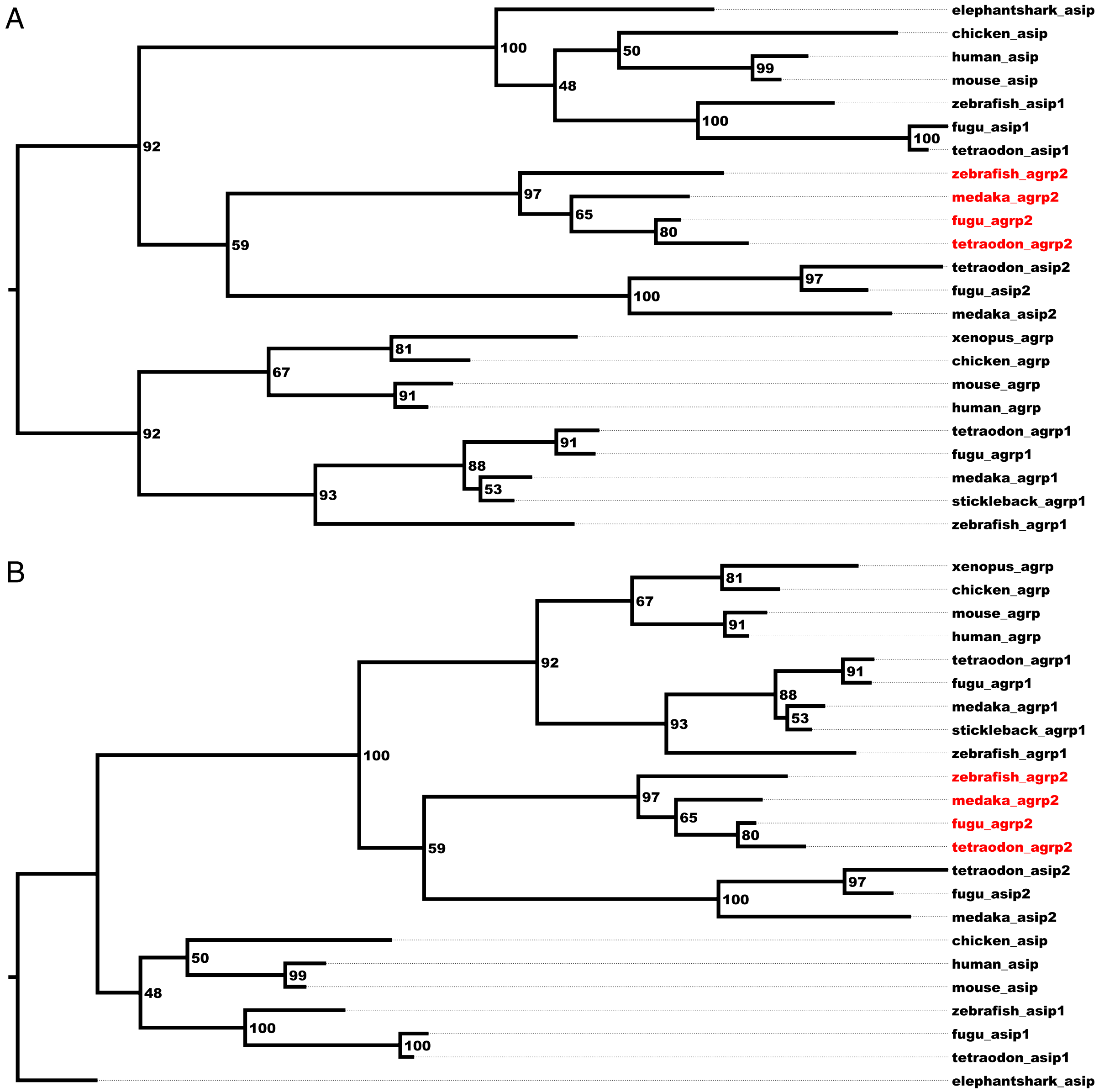

Fig. 1. Phylogeny of the agouti gene family members. The full-length sequences from Braasch and Postlethwait (1) were aligned (except sequences where the reference was to unspecified Contig regions) using MAFFT-EINSI 6.624b with default settings and 10 rebuilds. RAxML-III was used with the fast and easy settings: bootstrap maximum likelihood, Whelan and Goldman (WAG) model, estimate proportion of invariable sites, empirical base frequencies, and 100 bootstraps using easyrax.pl as interface. (*A*) This dendrogram is rooted on the *agrp* clusters. (*B*) This dendrogram is equivalent to *A* but rerooted on the elephant shark full-length sequence (*Callorhinchus milii*). The difference is that now the A2 cluster, containing the *agrp2* and *asip2* sequences, is grouped with the *agrp* cluster instead of the *asip* cluster. Bipartitions trees were viewed in Fig Tree 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/).

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