

Phylogeny and Biogeography of the Eleotrid Genus *Hypseleotris* (Teleostei: Gobioidi: Eleotridae), With Redescription of *H. cyprinoides*

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ABSTRACT. *Hypseleotris* includes eight described and four undescribed species of small, laterally compressed gobioid fishes, unusual among them in that they superficially resemble cyprinids. One species is widespread throughout the fresh and brackish waters of the old world southern hemisphere; the remaining eleven species form a radiation in Australia and southern New Guinea. In this study, morphological and molecular characters are combined in a total evidence phylogenetic analysis in order to investigate the patterns of diversification in Australian and other *Hypseleotris*, including a consideration of biogeography. The species *H. dayi*, *H. tohizonae*, *H. leuciscus*, and *H. guentheri* are synonymized with *H. cyprinoides*. Within Australian *Hypseleotris*, *H. klunzingeri* is the most basal taxon, and the remainder of the species fall into two groups: a radiation in Western Australia and the Northern Territory, and a second radiation in the eastern, central, and southeastern river drainages.

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Hypseleotris Gill, 1863 (carp gudgeons) is a small genus of eleotrid fishes, consisting of approximately twelve species, although confusion surrounds the status of some taxa. The genus is diagnosed by several morphological characters, including a strongly laterally compressed head and body, a small mouth not reaching the anterior border of the orbit, an elongate body cavity with several anal pterygiophores preceding the first vertebral hemal spine, and an ovoid blotch at the dorsal base of the pectoral fin. *Hypseleotris* species have a broad range, occurring from South Africa to Japan, through southeast Asia to Australia. The majority of species

are freshwater, although widespread taxa have some salt-water tolerance (Allen *et al.*, 2002). The most widespread species is *H. cyprinoides* (Cuvier & Valenciennes, 1837), recorded from southeast Asia to Japan and west to the Reunion Islands. Additional related taxa occur in South Africa and Madagascar, (*H. dayi* Smith, 1950 and *H. tohizonae* Steindachner, 1880, respectively), although Hoesé (1986) has suggested these may be conspecific with *H. cyprinoides*. Four species were described from the Philippines (Herre, 1927: *H. agilis*, *H. bipartita*, *H. modestus* and *H. pangel*); these are also considered by some (D.

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Table 1. Summary of valid *Hypseleotris* species as indicated by this study.

taxon	status
<i>H. agilis</i> Herre, 1927	synonym of <i>Ophieleotris aporos</i>
<i>H. aurea</i> (Shipway, 1950)	valid
<i>H. bipartita</i> Herre, 1927	synonym of <i>H. cyprinoides</i>
<i>H. compressa</i> (Krefft, 1864)	valid
<i>H. compressocephalus</i> Chen & Zheng, 1985	not <i>Hypseleotris</i>
<i>H. cyprinoides</i> (Cuvier & Valenciennes, 1837)	valid
<i>H. dayi</i> Smith, 1950	synonym of <i>H. cyprinoides</i>
<i>H. ejuncida</i> Hoese & Allen, 1983	valid
<i>H. galii</i> (Ogilby, 1898)	valid
<i>H. guentheri</i> (Bleeker, 1875)	synonym of <i>H. cyprinoides</i>
<i>H. hainanensis</i> Chen & Zheng, 1985	valid as <i>Neodontobutis hainanensis</i>
<i>H. kimberleyensis</i> Hoese & Allen, 1983	valid
<i>H. klunzingeri</i> (Ogilby, 1898)	valid
<i>H. leuciscus</i> (Bleeker, 1853)	synonym of <i>H. cyprinoides</i>
<i>H. modestus</i> Herre, 1927	synonym of <i>H. cyprinoides</i>
<i>H. pangel</i> Herre, 1927	possible synonym of <i>H. cyprinoides</i>
<i>H. raji</i> Herre, 1945	synonym of <i>Butis koilomatodon</i>
<i>H. regalis</i> Hoese & Allen, 1983	valid
<i>H. tohizonae</i> Steindachner, 1880	synonym of <i>H. cyprinoides</i>
<i>H. sp. 3</i> Murray-Darling (Unmack, 2000)	valid
<i>H. sp. 4</i> Midgley's (Larson & Hoese, 1996)	valid
<i>H. sp. 5</i> Lake's (Larson & Hoese, 1996)	valid
<i>H. n.sp.</i> Katherine River	valid

Hoese, personal communication) to be possible synonyms of *H. cyprinoides*. An additional species, *H. leuciscus* (Bleeker, 1853) was described from Sumatra, Indonesia, and is also probably synonymous with *H. cyprinoides* (Kottelat, 1993). The widespread species *H. guentheri* (Bleeker, 1875) is known from Indonesia, the Caroline Islands, New Caledonia, New Ireland and northern New Guinea. The remaining seven described and four undescribed species are found within Australian freshwaters. A summary of *Hypseleotris* species names is given in Table 1.

Within Australia, *Hypseleotris compressa* (Krefft, 1864) is the most widespread form, occurring from eastern Victoria north and west to the Pilbara region of Western Australia, as well as southern New Guinea. It also apparently can tolerate some salinity (Merrick & Schmida, 1984); populations sampled across coastal Queensland and the Northern Territory showed low levels of sequence divergence, and little population structure (McGlashan & Hughes, 2001). The remaining species have undergone two radiations, one in the northwestern portion of the Australia, the other in the southeast. Within northwestern Australia most species have narrow ranges, often being known from only one river, or just a small portion of a drainage (Allen *et al.*, 2002). *Hypseleotris aurea* (Shipway, 1950) is known from the Gascoyne and Murchison rivers in the southern Pilbara. *Hypseleotris kimberleyensis* Hoese & Allen, 1983 has been recorded from Manning Gorge, a tributary of the Fitzroy River and the nearby Calder River. *Hypseleotris regalis* Hoese & Allen, 1983 is found in the Roe and Prince Regent rivers while *H. ejuncida* Hoese & Allen, 1983 only occurs in the latter (Allen, 1982). A recently discovered undescribed species occurs in the upper parts of the Katherine River (H.K. Larson, pers. comm.). In contrast, the two described and three undescribed *Hypseleotris* species in southeastern Australia are all relatively widespread. Of these, Midgley's carp gudgeon (*Hypseleotris*

sp. 4; name used here follows Larson & Hoese, [1996], equivalent to *H. sp. A* [Allen, 1989] and *H. sp. 1* [Allen *et al.*, 2002]) is the most widespread, occurring in coastal drainages from the Tully River in northeastern Queensland to the Brisbane River, and the inland drainages including the entire Murray-Darling Basin, Bulloo River and Cooper Creek. *Hypseleotris klunzingeri* (Ogilby, 1898) has an identical distribution to Midgley's carp gudgeon within inland drainages and is also found from around Rockhampton in central coastal Queensland south to the Clarence River in northern New South Wales. Lake's carp gudgeon (*Hypseleotris sp. 5*; name used here follows Larson & Hoese, [1996], equivalent to *H. sp. B* [Allen, 1989] and *H. sp. 2* [Allen *et al.*, 2002]) only occurs in inland drainages (Murray-Darling Basin, Bulloo River and Cooper Creek). The fourth species found within the Murray-Darling Basin is the recently recognized, but undescribed Murray-Darling carp gudgeon (*Hypseleotris sp. 3*; Unmack, 2000; Allen *et al.*, 2002). The last species, *H. galii* (Ogilby, 1898), is restricted to coastal drainages from near Bundaberg in southeastern Queensland south to the Georges River, Sydney, New South Wales.

Most *Hypseleotris* species are poorly known, both in terms of their biology and their distribution and abundance. The exceptions to this pattern are the *Hypseleotris* from southeastern Australia. Here, *Hypseleotris* species are ubiquitous and usually dominate the freshwater fish fauna in terms of abundance and richness, with three or four species commonly being found sympatrically (Unmack, 2000). Even in disturbed habitats, they are often the only native fish to be found. Despite their abundance, surprisingly little is known of their biology, largely due to difficulties in distinguishing the species. Some work has been conducted on the reproductive biology of *H. klunzingeri* (Lake, 1967), *H. compressa* (Auty, 1978), *H. galii* (Anderson *et al.*, 1971; Mackay, 1975; Konagai & Rimmer, 1985). The identification,

natural habitat and reproduction of all southeastern endemic *Hypseleotris* spp. were reviewed in Unmack (2000).

This study was undertaken in order to clarify the taxonomy of *Hypseleotris* species and construct a phylogeny for the genus. This phylogeny is then used to explore patterns of morphological character evolution, and to frame consideration of the biogeographic patterns among *Hypseleotris* species, in particular the pattern of freshwater radiations in Australia.

Materials and methods

Morphological methods

Hypseleotris species in the collection of the Australian Museum were examined for external morphology with a stereomicroscope. Specimens of *H. compressa* (AMS I.22039-001), *H. cyprinoides* (AMS I.21896-004), *H. guentheri* (AMS I.7453), *H. klunzingeri* (AMS I.22898-001), *H. galii* (AMS I.19016-002), *H. aurea* (AMS I.25492-002), *H. sp. 4* (Midgley's) (AMS I.22097-006) and *H. sp. 5* (Lake's) (AMS I.17619-008) were cleared and double stained for bone (alizarin red) and cartilage (alcian blue) by the method of Pothoff (1984) and dissected by a modification of the method for small teleosts outlined in Weitzman (1974). Additional cleared and stained specimens of *H. compressa*, *H. klunzingeri*, *H. galii*, *H. regalis*, *H. sp. 4* (Midgley's), and *H. sp. 5* (Lake's) in the AMS collections were examined. Radiographs of *H. regalis*, *H. ejuncida*, and *H. kimberleyensis* were also prepared (see list below).

In addition to external and skeletal morphology, the fifth ceratobranchial toothplates of several species were examined with the scanning electron microscope. For the species *Hypseleotris compressa* (AMS I.38538-005), *H. cyprinoides* (AMS I.21896-004, AMS I.25047-001), *H. guentheri* (AMS I.7908), *H. klunzingeri* (AMS I.22898-001), *H. galii* (AMS I.29669-001), *H. sp. 4* (Midgley's: AMS I.34817-001) and *H. sp. 5* (Lake's: AMS I.16002-020), both the right and left toothplates were removed, dehydrated in 95% ethanol, air dried and mounted on stubs, then sputter coated with gold and examined on the LEO 457 VP scanning electron microscope at the Australian Museum, Sydney, following the protocol of Parenti & Thomas (1998).

Frozen or ethanol-preserved samples of *Hypseleotris* species for DNA analysis were derived from several sources. Specimens of *H. klunzingeri*, *H. sp. 4* (Midgley's), *H. sp. 5* (Lake's) and *H. sp. 3* (Murray-Darling) were collected by Peter Unmack from various localities in Queensland, New South Wales and Victoria, as detailed in Figure 1. Individuals of a new species from the Katherine River, Northern Territory, Australia, were collected by Dion Wedd, Helen Larson & J. Bywater. Samples of *H. compressa* were collected by Aarn at Townsville, Queensland, Australia. *Hypseleotris dayi* Smith, 1950 was collected in Beachwood Pond, Durban, South Africa, by Mike Coke. *Hypseleotris cyprinoides* tissue was provided by Takahiko Mukai, from a fish collected by Toshiyuki Suzuki at Iriomote Island, Okinawa Prefecture, Japan. Samples of wild-collected captive *H. tohizonae* Steindachner, 1880 in the Denver Zoo were provided by Rick Haeffner and Paul Earle. The specimens of *H. dayi*, *H. sp. 3* (Murray-Darling), and *H. n.sp.* Katherine River were also examined for morphological characters and radiographs were prepared. All specimens

used for DNA sequencing are vouchered in the Natural History Museum of Los Angeles County tissue collection, identified by species name, and, if more than one individual, by extract code (see list below).

The holotype of *H. leuciscus* (RMNH 4669) was examined and radiographed. For determination of species validity, the holotype of *H. raji* Herre, 1945 (CAS 139863) was also examined. The holotypes of *H. compressocephalus* Chen & Zheng, 1985 and *H. hainanensis* Chen & Zheng, 1985 were not examined; Chen *et al.* (2002) assign *H. hainanensis* to a new genus, *Neodontobutis*, and it is clear from the figure in Pan *et al.* (1991) that *H. compressocephalus* is not a species of *Hypseleotris*. Holotypes of *H. agilis*, *H. bipartita* and *H. pangal* are lost, presumably destroyed in World War II. For *H. guentheri*, types were not designated in the original description (Bleeker, 1875), but a specimen was located in the Natural History Museum, London, that corresponds to the description exactly. Although not designated a type by Bleeker (1875), and not given a catalogue number until recently, the specimen was shelved in the type collection and there is little doubt that this specimen is the same one referred to by Bleeker (1875). This specimen (BMNH 2003.8.7.1) was recorded as originating from "Oualan" (Bleeker, 1875) or "Rivers of Bourbon, of Benculen (Sumatra), and of Oualan" (Günther, 1861). Examination of the specimen data confirms that the locality was misread by these authors; the actual locality is Ovalau Island, Fiji. Types were not examined for *H. cyprinoides*, *H. dayi*, and *H. tohizonae*; instead, I relied on observations taken by Helen Larson (pers. comm.) for the first two species, and the detailed original description for the third.

Molecular methods

Sequence of the ND2 gene from *Philypnodon grandiceps* and *Eleotris sandwicensis* was obtained from GenBank (AF391458 and AF391477–78); a previous study (Thacker, 2003) showed that these two genera are closely related to *Hypseleotris*; its proximal sister group remains unknown. The species *H. ejuncida*, *H. regalis*, *H. kimberleyensis*, *H. leuciscus* and *H. guentheri* were not sequenced; only morphological characters were coded for these species.

Muscle tissue from each specimen was used for total genomic DNA extraction, performed with the QIAamp Tissue Kit (QIAGEN Inc., Chatsworth CA). PCR was performed using primer pairs GOBY L4919 (5'-CCCATACCCCGAAAATGATG-3') to GOBY H5513 (5'-GAGTAGGCTAGGATTTTWC GAAGYTG-3') and GOBY L5464 (5'-GGTTGAGGRGGCCTMAACCARAC-3') or the *Hypseleotris*-specific modification HYP5L5464 (5'-GGRTGAGGCGGRCTAAACCARAC-3') to GOBY H6064 (5'-CTCCTACTTAGAGCTTTGAAGGC-3') using Platinum Taq DNA polymerase (Invitrogen Corp., Rockville, MD), with a profile 94° for 1 min 30s, followed by 40 cycles of 94°/15s denaturation, 45–50°/45s annealing and 70°/1 min extension. These primers amplify, in two fragments, the complete mitochondrial ND2 gene (1047 base pairs). PCR products were electrophoresed on a 1.5% low melting point agarose gel (NuSieve GTG, Cambrex Bioproducts, Rockville ME), and bands were excised and cleaned with the QiaQuick gel extraction kit (QIAGEN Inc., Chatsworth CA). Using the same primers (1 µM rather than

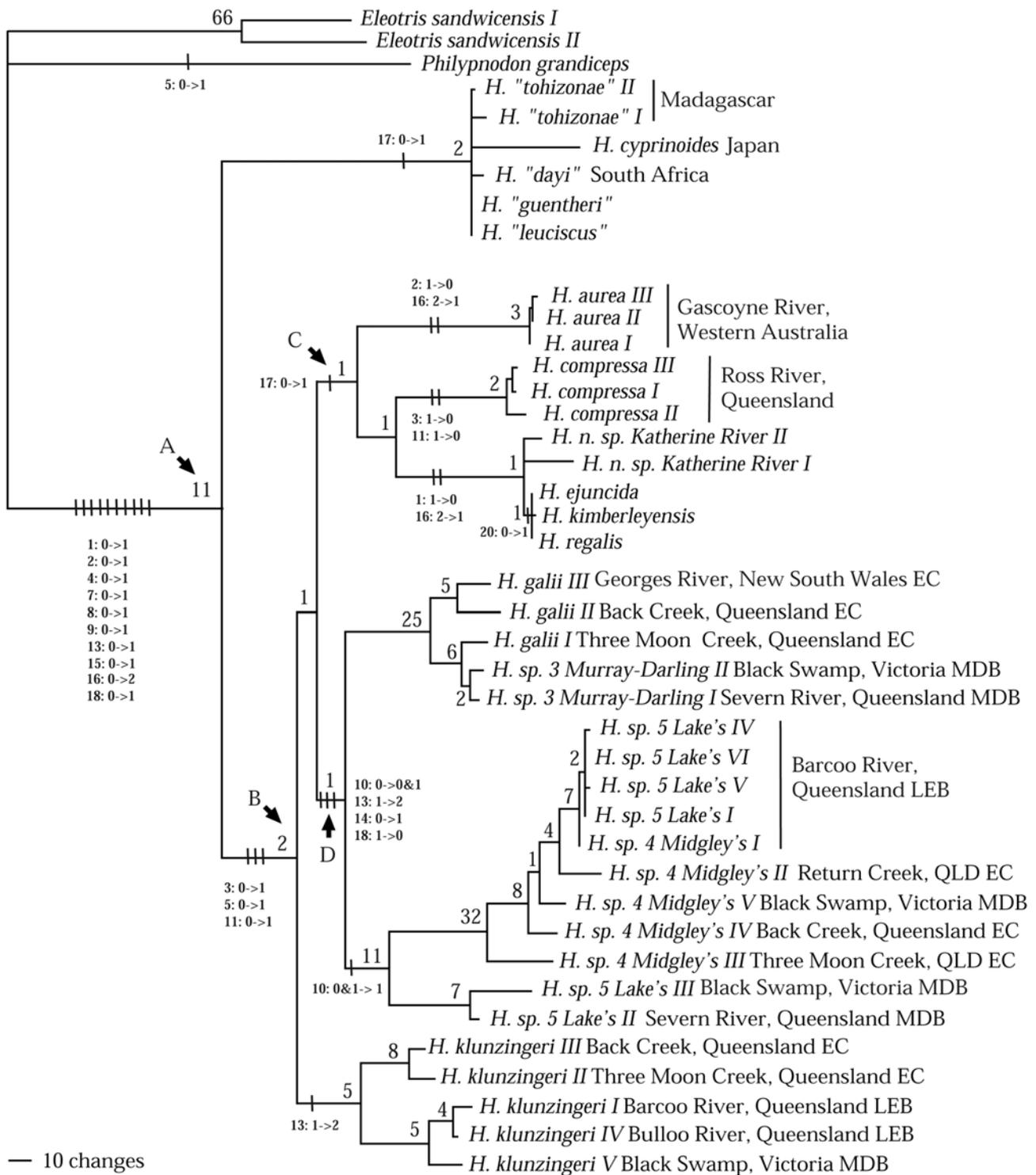


Fig. 1. Strict consensus of 189 most parsimonious hypotheses of *Hypseleotris* relationships. The numbers at nodes are decay index values, and changes of selected morphological characters are indicated with hash marks and the character number and state change (characters are represented with DELTRAN optimization). The species name and collection locality are indicated, and clades discussed in the text are lettered. In the southeastern species, abbreviations after the name and locality indicate the province or drainage basin: East Coast (EC, also known as Eastern Province), Murray-Darling Basin (MDB, Murray-Darling Province), or Lake Eyre Basin (LEB, Central Australian Province).

10 µM) the PCR fragments were cycle sequenced using the Big Dye terminator/Taq FS ready reaction kit and run on an ABI 377 automated sequencer (Perkin-Elmer, Foster City, CA). The heavy and light strands were sequenced separately. The resultant chromatograms for the heavy and light strands were reconciled using Sequencher (GeneCodes Corp., Ann Arbor, MI) to check basecalling, translated to amino acid sequence using the “mammalian mtDNA” code, concatenated for each taxon, and aligned by eye. Aligned nucleotide sequences were exported as NEXUS files from Sequencher, and imported into MacClade (Maddison & Maddison, 2000), where morphological data were added, using the “extended standard” character state option. Phylogenetic analyses were performed using PAUP*, version 4.0b8 (Swofford, 2001). One thousand replications of a heuristic search were performed, with *Eleotris sandwicensis* and *Philypnodon grandiceps* designated as the outgroup taxa. Decay indices (Bremer, 1988) were calculated with PAUP* and TreeRot v.2 (Sorenson, 1999).

Specimens examined

Identification of species follows the conclusions of this study: *Hypseleotris cyprinoides* includes *H. dayi*, *H. tohizonae*, *H. guentheri*, and *H. leuciscus*. *Hypseleotris* taxa are listed first, followed by outgroup taxa. Institutional abbreviations are as follows: AMS (Australian Museum, Sydney); BMNH (Natural History Museum, London); LACM (Natural History Museum of Los Angeles County); RMNH (Rijksmuseum van Natural Historie, Leiden).

Hypseleotris aurea AMS I.22743-001 (4), AMS I.25492-002 (43+3C&S), AMS I.25493-003 (1), Murchison River, Western Australia, Australia; AMS I.26406-003 (4), Wittenoom Gorge, Western Australia, Australia.

Hypseleotris compressa AMS I.15903-009 C&S no 367, Clarence River, New South Wales, Australia; AMS I.22039-001 (44+3C&S), Cairns, Queensland, Australia; AMS I.22088-003 C&S no 36 Burdekin River, Queensland, Australia; AMS I.22699-004 C&S no 354, Daintree River, Queensland, Australia; AMS I.22710-004 C&S no 352, Cape Tribulation, Queensland, Australia; AMS I.29681-001 (13) Nhulunbuy, Northern Territory, Australia; AMS I.35747-008 (86), Creek east of Grafton, New South Wales, Australia; AMS I.38538-005 (41), Ninds Creek, Queensland, Australia; AMS I.38557-002 (24), Scrubby Creek, Queensland, Australia; AMS I.38593-005 (9), Kandanga Creek, Queensland, Australia; AMS I.40090-006 (22), AMS I.40090-007 (64), Burdekin River, Queensland, Australia; AMS I.40328-005 (39), Mortons Creek and Hastings River, New South Wales, Australia.

Hypseleotris cyprinoides AMS IB.496 (1), Fiji; AMS IB.2246 (1), Hienghene River, New Caledonia; AMS I.7244 (1), Samoa; AMS I.7453(2), AMS I.7454 (2 C&S), Tavua, Fiji; AMS I.7906 (1), AMS I.7907 (1), AMS I.7908 (1), AMS I.7909 (1), AMS I.7910 (1), AMS I.7911 (1), AMS I.7912 (1), AMS I.7913 (1), Apia, Samoa; AMS I.19656-005 (2), Babelthup Island, Palau, Caroline Islands; AMS I.21896-004 (4+3C&S), Tobuan Creek, Lingayan Bay, Philippines; AMS I.21924-002 (3), Batangas Province, Philippines; AMS I.25047-001 (4), Lagonoy River, Philippines; BMNH 1926.3.6.96-102 (12), Apia, Samoa; BMNH 1965.3.9.5 (1), Inawi, St. Joseph River, New Guinea; BMNH 2003.8.7.1 (1), Ovalau, Fiji (holotype of *H.*

guentheri); LACM 51857.003 (94), LACM 51858.010 (17), Guadalcanal, Solomon Islands; RMNH 4669 (1), West Sumatra (holotype of *H. leuciscus*).

Hypseleotris ejuncida AMS I.22745-001 (4) x-rayed, Gundarara Creek, 2 km above junction with Prince Regent River, Prince Regent Reserve, West Kimberley, Western Australia, Australia.

Hypseleotris galii AMS I.19016-002 (17+3 C&S), Poperaperin Creek, New South Wales, Australia; AMS I.19899-001 (7), Lane Cove River, New South Wales, Australia; AMS I.27273-001 C&S no 391, Saleyard Creek, New South Wales, Australia; AMS I.29669-001 (96), Creek between Cooluna and Noosa, Queensland, Australia; AMS I.35608-003 (42), Coffs Harbour, New South Wales, Australia; AMS I.38590-002 (8), Gillens Creek, Queensland, Australia; AMS I.40331-007 (8), Dungay Creek, New South Wales, Australia; AMS I.40350-004 (7), Clarence River, New South Wales, Australia.

Hypseleotris kimberleyensis AMS I.22742-001 (3) x-rayed, AMS I.25529-007 (2) x-rayed, Barnett River near Barnett Gorge, Central Kimberley, Western Australia.

Hypseleotris klunzingeri AMS IB.872 (8), Murray River, New South Wales, Australia; AMS I.13458 (12), Narrandera, North Yanco, New South Wales, Australia; AMS I.14963 (23), Colombo Creek, New South Wales, Australia; AMS I.17616-001 C&S no 345, Yanga Creek, Murrumbidgee River System, New South Wales, Australia; AMS I.17618-003 (133), Lake Bonney, South Australia, Australia; AMS I.22898-001 (86+3C&S), Mary River, Tinana Creek, Queensland, Australia; AMS I.29930-001 (34), Namoi River, New South Wales, Australia; AMS I.35588-006 (5), Meehi River, New South Wales, Australia; AMS I.38592-008 (106), Yabba Creek, Queensland, Australia; AMS I.39059-006 (27), Tuppal Creek, New South Wales, Australia; AMS I.40066-004 (19), Warrego River, Queensland, Australia.

Hypseleotris regalis AMS I.22744-001 (4) x-rayed, AMS I.22744-002 C&S no 368 Wyulda Creek, 2 km above junction with Roe River, Prince Regent Reserve, West Kimberley, Western Australia, Australia.

Hypseleotris sp. 4 (Midgley’s) AMS I.18072-001 (4), north of Bonshaw, Queensland, Australia; AMS I.18965-009 C&S no 369, Namoi drainage, Wanourie Creek, New South Wales, Australia; AMS I.21284-005 C&S no 361, Montrose Creek, Queensland, Australia; AMS I.22097-006 (25+3C&S), Granite Creek, Queensland, Australia; AMS I.27257-002 C&S no 357, Barwon River and Namoi River Junction, New South Wales, Australia; AMS I.29929-001 (9), Northern River, New South Wales, Australia; AMS I.34802-001 (11), Bremer River, South Australia, Australia; AMS I.34816-001 (26), Angas River, Lake Alexandrina, South Australia, Australia; AMS I.34817-001 (24), Dawson Creek, Angas River, South Australia, Australia; AMS I.40163-001 (56), Creek off Peel River, New South Wales, Australia; AMS I.40164-006 (16), Darling River, New South Wales, Australia; AMS I.40489-001 (7), Macquarie Marshes, Macquarie River, New South Wales, Australia.

Hypseleotris sp. 5 (Lake’s) AMS I.16002-020 (12), Darling River, New South Wales, Australia; AMS I.17619-008 (4+3C&S), Lake Bonney, South Australia, Australia; AMS I.27257-003 C&S no 358, AMS I.27257-004 C&S no 359, Barwon River and Namoi River Junction, New South Wales, Australia; AMS I.37820-010 (10), AMS

Table 2. Matrix of morphological character states used in this study. Character descriptions are given in the text.

taxon	character																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>H. agilis</i>	1	1	1	1	0	1	1	1	1	0	0	1	1	0	1	0	0&1	0	0	0
<i>H. aurea</i>	1	0	0	1	1	0	1	1	1	0	1	0	1	0	1	1	1	1	1	0
<i>H. compressa</i>	1	1	1	1	1	1	1	1	1	0	0	0&1	1	0	1	2	1	1	1	0
<i>H. cyprinoides</i>	1	1	1	1	0	1	1	1	1	0	0	0	1	0	1	2	1	1	0	0
<i>H. dayi</i>	?	?	1	1	0	1	1	1	1	0	0	1	1	0	1	2	1	1	0	0
<i>H. ejuncida</i>	0	1	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1
<i>H. galii</i>	1	1	0	1	1	1	1	1	1	0&1	1	0&1	2	1	1	2	0	0	0	0
<i>H. guentheri</i>	1	1	1	1	0	1	1	1	1	0	0	1	1	0	1	2	1	1	0	0
<i>H. kimberleyensis</i>	0	1	0	1	1	2	1	1	1	0	1	1	1	0	1	1	1	1	1	1
<i>H. klunzingeri</i>	1	1	0	1	1	1	1	1	1	0&1	1	0&1	2	0&1	1	2	0	1	0	0
<i>H. leuciscus</i>	?	1	1	1	0	1	1	1	1	0	0	1	1	0	1	2	1	1	0	0
<i>H. regalis</i>	0	1	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	1
<i>H. tohizonae</i>	?	1	?	1	0	1	1	1	1	0	0	1	1	0	?	?	?	1	0	0
<i>H. sp. 3 Murray-Darling</i>	1	1	0	1	1	0	1	1	1	0&1	1	0&1	2	0&1	1	2	0	0	0	0
<i>H. sp. 4 Midgley's</i>	1	1	0	1	1	0	1	1	1	0&1	1	0&1	2	1	1	2	0	0	0	0
<i>H. sp. 5 Lakes</i>	1	1	0	1	1	2	1	1	1	1	1	0&1	2	1	1	2	0	0	1	0
<i>H. n.sp. Katherine River</i>	0	1	0	1	1	0	1	1	1	0	1	1	1	0	1	1	1	1	1	0
<i>Philypnodon grandiceps</i>	0	0	0	0	1	0&2	0	0	0	0&1	0&1	1	0	0	0	0	0	0	1	0
<i>Eleotris sandwicensis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

I.37820-012 (3), AMS I.39060-002 (74), Tuppal Creek, New South Wales, Australia; AMS I.40339-001 (60), Dawson River, Queensland, Australia.

Eleotris sandwicensis LACM 56053-1 (1), LACM 56053-2 (1), North Oahu, Hawaii.

Philypnodon grandiceps AMS I.31622-001 (5), Nymboida River, New South Wales, Australia; AMS I.34350-004 (9), Werribee Creek, Queensland, Australia; AMS I.39060-006 (12), Tuppal Creek, New South Wales, Australia; LACM 4261-3 (16), MacDonald River, New South Wales, Australia. Radiographs only of SU 5797 (3) and USNM 48820 (4) were also examined.

In addition to these preserved specimens, frozen specimens of *H. dayi*, *H. sp. 3* (Murray-Darling) and *H. n.sp.* Katherine River were examined and radiographed. Individuals used for DNA sequencing are uncatalogued in the tissue collection of the Natural History Museum of Los Angeles County. Specimens used are identified by species name and extract number: *H. aurea* HAURI, HAURII, HAURIII; *H. compressa* HCOMI, HCOMII, HCOMIII; *H. cyprinoides* HCYP; *H. dayi* HDAY; *H. tohizonae* HTOHI, HTOHII; *H. galii* HGALI, HGALII, HGALIII; *H. klunzingeri* HKLUI, HKLUII, HKLUIII, HKLUIV, HKLUV; *H. n.sp.* Katherine River HKATI, HKATII; *H. sp. 3* (Murray-Darling) HMDI, HMDII; *H. sp. 4* (Midgley's) HMIDI, HMIDII, HMIDIII, HMIDIV, HMIDV; *H. sp. 5* (Lake's) HLAKI, HLAKII, HLAKIII, HLAKIV, HLAKV, HLAKVI.

Results

The morphological characters are numbered in accordance with the character matrix, shown in Table 2. Examination of internal anatomy from cleared and stained specimens or radiographs did not reveal any characters useful for resolving relationships among most of the species. Although slight variations were present in the robustness of bones, squamation and dentition, no significant differences were observed. Examination of fifth ceratobranchial toothplates

did not yield any characters useful for resolving phylogenetic relationships. The toothplates feature smooth, sharp teeth, evenly distributed across the toothplate except for a slightly denser region at the anterolateral edge. Tooth size was also generally uniform, with the exception of enlarged teeth on each toothplate along the posteriomedial borders.

- Sexual dimorphism, with pronounced enlargement of the dorsal head/nape region in males. 0: no dimorphism in head/nape shape; 1: dimorphism present, males with enlarged heads/napes. The enlarged head/nape in males (state one) is seen in *H. cyprinoides*, *H. guentheri*, *H. compressa*, *H. aurea*, *H. galii*, *H. klunzingeri*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's). The males of *H. ejuncida*, *H. kimberleyensis*, *H. regalis*, *H. n.sp.* Katherine River and outgroups exhibit state zero. The condition of this character is unknown in *H. dayi*, *H. tohizonae* and *H. leuciscus*. In some species (*H. aurea*, *H. cyprinoides* and *H. guentheri*) the enlargement is less prominent than others.
- Sexual dimorphism, elongated posterior rays in dorsal and anal fin, particularly during the breeding season. 0: no dimorphism; 1: dimorphism present, males with elongated dorsal- and anal-fin rays, extending to the caudal peduncle. The elongated fins in males (state one) are seen in *H. leuciscus*, *H. cyprinoides*, *H. tohizonae*, *H. guentheri*, *H. compressa*, *H. galii*, *H. klunzingeri*, *H. sp. 4* (Midgley's), *H. sp. 5* (Lake's), *H. sp. 3* (Murray-Darling), *H. ejuncida*, *H. kimberleyensis*, *H. regalis* and *H. n.sp.* Katherine River. The fins are not elongate in males (state zero) in outgroups and *H. aurea*; the condition of this character is unknown in *H. dayi*. The possibility remains that the absence of elongate fins in some species is an artifact due to collection outside the breeding season. Collections that are more seasonally complete will be necessary to address this.

- 3 Sensory canal pores. 0: preopercular (3–4) and suborbital (2) canal pores absent; 1: pores present. Sensory canal pores are absent (state zero) in outgroups, *H. aurea*, *H. ejuncida*, *H. kimberleyensis*, *H. regalis*, *H. n.sp.* Katherine River, *H. galii*, *H. klunzingeri*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's). Pores are present (state one) in *H. compressa*, *H. cyprinoides*, *H. dayi*, *H. guentheri*, and *H. leuciscus*. The condition of this character is not known for *H. tohizonae*. Polarity of this character is given based on assigning state zero to the outgroups used; the more general condition among eleotrids is for these pores to be present.
- 4 Pigment blotch on dorsal portion of pectoral-fin base. 0: blotch absent; 1: blotch present. A distinct, dark, oblong blotch on the pectoral-fin base is present (state one) in all species of *Hypseleotris*, and absent (state zero) in outgroups. There is some spotting and mottling on the pectoral-fin bases of *Eleotris sandwicensis*, but not a distinct single blotch as in *Hypseleotris*.
- 5 Interorbital scales. 0: interorbital scales present; 1: interorbital scales absent. Interorbital scales are present (state zero) in *Eleotris sandwicensis*, *Hypseleotris cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus* and *H. guentheri*. The scales are absent (state one) in *Philypnodon grandiceps* and all other species of *Hypseleotris*.
- 6 Squamation on nape. 0: nape scaled, scales cycloid, small, embedded; 1: nape scaled, scales large, overlapping, cycloid or ctenoid; 2: no scales on nape. The nape is scaled with small, embedded cycloid scales (state zero), in *H. aurea*, *H. ejuncida*, *H. regalis*, *H. n.sp.* Katherine River, *H. sp. 3* (Murray-Darling) and *H. sp. 4* (Midgley's). The nape is scaled with large, overlapping cycloid or ctenoid scales (state one) in *H. cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus*, *H. compressa*, *H. guentheri*, *H. galii* and *H. klunzingeri*. The nape is unscaled (state two) in *H. sp. 5* (Lake's) and *H. kimberleyensis*. Outgroup taxon *Eleotris sandwicensis* exhibits state zero, and *Philypnodon grandiceps* is polymorphic for states zero and two.
- 7 Laterally compressed, narrow head, much taller than wide. 0: head not compressed; 1: head compressed. The head is compressed (state one) in all species of *Hypseleotris*, and not compressed (state zero) in outgroups.
- 8 Pectoral-fin base size. 0: large, with dorsal most ray just behind or slightly below upper attachment of gill membrane; 1: small, with dorsal most ray well below upper attachment of gill membrane. The pectoral-fin base is small (state one) in all species of *Hypseleotris*, and large (state zero) in outgroups.
- 9 Mouth shape. 0: mouth not small, premaxilla extending posteriad past anterior edge of eye; 1: small terminal mouth, premaxilla not extending past anterior edge of eye. The mouth is small and terminal (state one) in all species of *Hypseleotris*, and large (state zero) in outgroups.
- 10 First dorsal-fin spines. 0: six spines; 1: seven or more spines. Six spines (state zero) is found in *H. compressa*, *H. cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus*, *H. guentheri*, *H. aurea*, *H. ejuncida*, *H. kimberleyensis*, *H. regalis*, *H. n.sp.* Katherine River and *Eleotris sandwicensis*. Both states are seen in *Philypnodon grandiceps*, *H. galii*, *H. klunzingeri*, *H. sp. 3* (Murray-Darling) and *H. sp. 4* (Midgley's). *Hypseleotris sp. 5* (Lake's) always has more than six spines (state 1).
- 11 Second dorsal-fin elements. 0: eight or nine elements; 1: ten to fourteen elements. The second dorsal fin consists of one spine followed by seven or more soft segmented rays. The most posterior ray is split to its base but counted as a single element. *Hypseleotris compressa*, *H. cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus*, *H. guentheri* and *Eleotris sandwicensis* have eight or nine elements (state zero); the species *H. aurea*, *H. galii*, *H. klunzingeri*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's), *H. sp. 5* (Lake's), *H. ejuncida*, *H. kimberleyensis*, *H. regalis* and *H. n.sp.* Katherine River have second dorsal fins with ten to fourteen elements (state one). The outgroup taxon *Philypnodon grandiceps* is polymorphic, exhibiting state zero or one. The distinction between states given here was used because in no *Hypseleotris* species does the dorsal-fin ray range span that breakpoint (no *Hypseleotris* species is polymorphic for this character).
- 12 Interneural gap in dorsal fin. 0: interneural gap absent; 1: interneural gap present. An interneural gap, which is an interneural space between the first and second dorsal fins that does not have a pterygiophore associated with it, is absent (state zero) in *H. aurea*, *H. cyprinoides* and *Eleotris sandwicensis*. The gap is present (state one) in *Philypnodon grandiceps*, *H. dayi*, *H. tohizonae*, *H. leuciscus*, *H. guentheri*, *H. regalis*, *H. kimberleyensis*, *H. ejuncida* and *H. n.sp.* Katherine River. The interneural gap is seen uncommonly (coded as state 0&1) in *H. compressa*, *H. galii*, *H. klunzingeri*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's), species in which the dorsal pterygiophore patterns are variable.
- 13 Second dorsal-fin pigment in males. 0: Second dorsal fin clear to dusky, or with black bands on pale ground, not brightly coloured; 1: Basal third to half of second dorsal fin with light spots on black ground, then with distal stripes; 2: Second dorsal fin unspotted and with three to five coloured lateral bands, generally (from body dorsad) pale, dark, orange, white. The second dorsal is without a spotted or striped pattern in *Eleotris sandwicensis*, and with a darkly striped pattern unlike that seen in *Hypseleotris* in *Philypnodon grandiceps*; both are coded as state zero. The second dorsal is spotted (state one) in *H. compressa*, *H. cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus*, *H. guentheri*, *H. aurea*, *H. regalis*, *H. kimberleyensis*, *H. ejuncida* and *H. n.sp.* Katherine River. The second dorsal is striped (state two) in *H. galii*, *H. klunzingeri*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's). This character was coded in part from descriptions and

photos of live colouration in Hoese & Allen (1983); Allen (1991); and Unmack (2000); this colouration is most intense during the breeding season.

- 14 Anal-fin element number. 0: nine to twelve elements; 1: thirteen, fourteen or fifteen elements. The anal fin consists of a single spine, followed by nine to fourteen soft rays. Nine to twelve anal elements (state zero) are found in outgroups, *H. cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus*, *H. compressa*, *H. guentheri*, *H. aurea*, *H. regalis*, *H. ejuncida*, *H. kimberleyensis* and *H. n.sp.* Katherine River. Thirteen to fifteen elements (state 1) are found in *H. galii*, *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's). *Hypseleotris klunzingeri* and *H. sp. 3* (Murray-Darling) exhibit both states. The breakpoint between states for this character was chosen to minimize the number of *Hypseleotris* species coded as polymorphic.
- 15 Anal-fin elements preceding first hemal spine (elongate body cavity). 0: 0–5 anal-fin elements preceding first hemal spine; 1: 6–11 anal-fin elements preceding the first hemal spine. Six or more anal-fin elements precede the first hemal spine (state 1) in all species of *Hypseleotris* examined; the character state is unknown for *H. tohizonae*. In *Hypseleotris* species, this character is correlated with a body cavity that is very elongate posteriad. Outgroups have five or fewer anal elements preceding the first hemal spine (state zero). The breakpoint between states (counts of anal-fin elements preceding first hemal spine) was determined by coupling count information with the observation that *Hypseleotris* species also have the elongate body cavity.
- 16 Precaudal vertebrae. 0: fewer than 13; 1: 13; 2: more than 13. The condition of fewer than 13 precaudal vertebrae (state zero) is found in the outgroups. Thirteen precaudal vertebrae (state one) are present in *H. aurea*, *H. ejuncida*, *H. kimberleyensis*, *H. regalis* and *H. n.sp.* Katherine River. More than thirteen precaudal vertebrae (state 2) are present in *H. cyprinoides*, *H. dayi*, *H. leuciscus*, *H. compressa*, *H. guentheri*, *H. galii*, *H. klunzingeri*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's). The condition for this character is unknown for *H. tohizonae*.
- 17 Caudal vertebrae. 0: 13–19; 1: 9–12. Thirteen to nineteen caudal vertebrae (state zero) is observed in *H. galii*, *H. klunzingeri*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's), *H. sp. 5* (Lake's) and outgroups. Nine to twelve caudal vertebrae (state 1) is seen in *H. cyprinoides*, *H. dayi*, *H. leuciscus*, *H. compressa*, *H. guentheri*, *H. aurea*, *H. ejuncida*, *H. kimberleyensis*, *H. regalis* and *H. n.sp.* Katherine River. The condition of this character is unknown for *H. tohizonae*.
- 18 Caudal-fin pigment pattern: dark blotch in ventral half of fin, just posteriad of caudal peduncle. 0: caudal blotch absent; 1: caudal blotch present. A blotch on the ventral half of the caudal fin (state one) is seen in *H. cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus*, *H. guentheri*, *H. compressa*, *H. aurea*, *H. ejuncida*, *H. kimberleyensis*, *H. regalis* and *H. klunzingeri*. *Hypseleotris galii*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's), *H. sp. 5* (Lake's), and outgroups lack the caudal blotch (state zero).
- 19 Scales on cheek. 0: scales present on cheek; 1: cheek not scaled, or with few tiny embedded cycloid scales. The cheek is scaled (state zero) in *Eleotris sandwicensis*, *H. cyprinoides*, *H. dayi*, *H. galii*, *H. guentheri*, *H. klunzingeri*, *H. leuciscus*, *H. tohizonae*, *H. sp. 3* (Murray-Darling), and *H. sp. 4* (Midgley's). The cheek is naked or with very few scales (state 1) in *Philypnodon grandiceps*, *H. sp. 5* (Lake's), *H. aurea*, *H. compressa*, *H. regalis*, *H. ejuncida*, *H. kimberleyensis* and *H. n.sp.* Katherine River.
- 20 Slender body. 0: body not slender, depth at anal-fin origin more than 20% of standard length in males; 1: body slender, depth at anal-fin origin less than 20% of standard length in both males and females. A slender body (state 1) in both sexes is found in *H. regalis*, *H. ejuncida* and *H. kimberleyensis*. Other *Hypseleotris* species and outgroups share state zero. Some data for this character were taken from Hoese & Allen (1983).

Some autapomorphic characters were identified that are useful for species identification, but not informative for phylogenetic analysis. The species *H. klunzingeri* may be distinguished by the presence of transverse rows of papillae on the face; females of *H. galii* possess a black urogenital papilla; and *H. aurea* has notably small scales (34–43 in lateral series).

Of the 1047 base pairs of the ND2 gene, 428 were parsimony-informative (sequences available in GenBank under accession numbers AF514365–AF514396). All morphological characters were informative, for a total of 448 informative characters in the combined data set. Parsimony analysis of the combined molecular and morphological data set resulted in 189 most parsimonious hypotheses, with length 1488, consistency index 0.586, retention index 0.803 and rescaled consistency index of 0.471; a strict consensus of these hypotheses is shown in Figure 1. If the data are analysed without the five species for which molecular data are lacking (*Hypseleotris ejuncida*, *H. regalis*, *H. kimberleyensis*, *H. leuciscus* and *H. guentheri*), the single most parsimonious topology that results is the same as for the combined analysis, but with those taxa pruned out, with one slight difference. In the DNA only tree, there is structure in the clade containing *H. cyprinoides*, of the form: (*H. cyprinoides* [*H. tohizonae I* (*H. dayi*, *H. tohizonae II*)]). In the strict consensus of trees from the combined analysis, these taxa form a polytomy.

Discussion

Basal *Hypseleotris*

The phylogenetic hypothesis presented in Figure 1 supports (with a decay index of eleven) a monophyletic *Hypseleotris* (clade A). The genus is diagnosed by two sexually dimorphic characters, the presence of enlarged heads in males (reversed in *H. ejuncida*, *H. kimberleyensis*, *H. regalis* and *H. n.sp.* Katherine River), and the presence of elongated dorsal and anal fins in males (reversed in *H. aurea*); the presence of a distinct pigment blotch on the pectoral-fin base; a constricted pectoral-fin base; a posteriorly elongate body cavity, with 6–11 anal pterygiophores preceding the first hemal spine; a precaudal vertebral count of 13 or greater; a strongly laterally compressed head and body; a small, terminal mouth whose posterior border does not reach the anterior border of the eye; a blotch of pigment on the ventral half of the caudal peduncle (reversed in *H. galii*, *H. sp. 3* [Murray-Darling], *H. sp. 4* [Midgley's], and *H. sp. 5* [Lake's]) and, a change in second dorsal-fin pigment to a pattern of distal stripes and proximally pale dots on black ground. The most basal clade contains the nominal taxa *H. cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus* and *H. guentheri*. The remaining clade contains all the Australian species.

Several conclusions regarding the identity of species and the allocation of names are supported by the phylogenetic hypothesis. In addition to the well-known taxa, other names have been used for *Hypseleotris* species that are not *Hypseleotris*. Two species from China, *H. compressocephalus* (Chen, 1985) and *H. hainanensis* Chen, 1985, lack the diagnostic features of the genus; both lack the pectoral-fin pigment blotch, the small, terminal mouth and the striped/spotted second dorsal-fin pattern. Additionally, *H. compressocephalus* has dorsal fin element counts of IX+I,13, higher than any *Hypseleotris* except *H. sp. 5* (Lake's) (Pan *et al.*, 1991). Chen *et al.* (2002) confirm that *H. hainanensis* is not *Hypseleotris*, describing for it a new genus, *Neodontobutis*. The species described as *H. raji* Herre, 1945 is also not *Hypseleotris*; it also lacks the pectoral-fin pigment blotch, the small, terminal mouth and the striped/spotted second dorsal-fin pattern, and the series of anal-fin pterygiophores preceding the first hemal spine. The locality for the *H. raji* holotype (CAS 139863) is listed as the Adyar River, Madras, S. India, in the records of the California Academy of Sciences. However, in the description the locality is listed as a brook near Un Long, New Territory, Hong Kong. Doug Hoese (pers. comm.) regards *H. raji* as a synonym of *Butis koilomatodon* (Bleeker, 1849).

Two issues surrounding the non-Australian species were considered: the number and identity of species found in the Philippines, and the validity of several taxa (*H. dayi*, *H. tohizonae*, *H. leuciscus* and *H. guentheri*) relative to *H. cyprinoides*. Herre (1927) lists five Philippine species of *Hypseleotris*: *H. cyprinoides*, *H. modestus*, *H. agilis*, *H. bipartita* and *H. pangel*. Herre (1927) doubted the presence of *H. cyprinoides* in the Philippines, but it has since been confirmed to exist there. Herre (1927) distinguishes *H. modestus* from *H. cyprinoides* only in sometimes having one more soft dorsal ray and one to two more anal rays. Kottelat *et al.* (1993) indicated that *H. modestus* is a

synonym of *H. leuciscus*, itself possibly a synonym of *H. cyprinoides*. Examination of the type of *H. leuciscus* in this study (RMNH 4669) shows that *H. leuciscus* (and thus *H. modestus*) is synonymous with *H. cyprinoides*, as discussed below and shown in Table 1.

The other three species considered by Herre (1927: *H. agilis*, *H. bipartita* and *H. pangel*) are known only from the Philippines. Types of these species are not extant, and are presumed to have been destroyed in World War II (Eschmeyer, 1998). Based on Herre (1927), who distinguishes them by slight differences in body proportions and by their colour patterns, all three species have meristic values consistent with *H. cyprinoides*. *Hypseleotris agilis* is the most distinctive, featuring a pattern of eight spots mediolaterally and three stripes radiating posteriad from the eye, with a fourth crossing the opercle but not extending to the eye. Specimens of *Ophieleotris aporos* captured at Laguna de Bay, near Manila, fit the description of *H. agilis*, and therefore we regard *H. agilis* as a synonym of *O. aporos*.

The two remaining Philippine species of Herre (1927) are *H. bipartita* and *H. pangel*; the specimens Herre (1927) examined for these species fall into two size classes. Those classified as *H. bipartita* ranged from 22 to 37 mm in length and exhibited a spot or short bar on the ventral portion of the caudal-fin base, and a second dorsal in males with pale spots on a black background. The *H. pangel* specimens were 32 to 47 mm in length and featured the second dorsal, anal and caudal fins with blotches or bars, and in some specimens, a fine lateral black band. The description and illustration of *H. bipartita* coincides very well with that of *H. cyprinoides*, that of *H. pangel* less so, but the *H. pangel* specimens are larger and variations in colour pattern may develop with size. Given the similarity in colour pattern and absence of unique differences, we recommend synonymizing *H. bipartita* with *H. cyprinoides*. We reserve judgment on *H. pangel*; if the colouration differences reported by Herre (1927) are not ontogenetically variable, then it may be a valid species. We have no specimens of *H. pangel*, therefore, we do not consider it further.

The issue of species identities throughout the range of non-Australian *Hypseleotris* is largely a question of the boundaries and variability within *H. cyprinoides*. This widespread species of *Hypseleotris* can tolerate salt water (Bruton, 1996), and is known from South Africa, Madagascar, Reunion Island, east to New Guinea and north to the Philippines and Japan. The names *H. dayi* (South Africa), *H. tohizonae* (Madagascar), *H. leuciscus* (Indonesia and west Pacific) and *H. guentheri* (northern New Guinea and west Pacific) have all been used for fish that agree with the description of *H. cyprinoides*, with some minor variation in colour pattern. The phylogenetic analysis in this study included morphological data for *H. cyprinoides*, *H. dayi*, *H. tohizonae*, *H. leuciscus* and *H. guentheri*, coupled with DNA sequence for *H. cyprinoides*, *H. dayi* and *H. tohizonae*; these five taxa formed a polytomy in the total evidence analysis. Based on these results we advocate synonymization of *H. dayi*, *H. leuciscus*, *H. tohizonae* and *H. guentheri* under *H. cyprinoides*. This recommendation is in accord with similar remarks by Hoese (1986) for *H. dayi* and *H. tohizonae*, and Kottelat *et al.* (1993) for *H. leuciscus* (Table 1). *Hypseleotris cyprinoides* is formally rediagnosed below.

Redescription of *Hypseleotris cyprinoides*

Hypseleotris cyprinoides (Valenciennes in Cuvier & Valenciennes, 1837: 248).

Synonyms. *Hypseleotris bipartita* Herre, 1927; *H. dayi* Smith, 1950; *H. guentheri* (Bleeker, 1875); *H. leuciscus* (Bleeker, 1853); *H. tohizonae* (Steindachner, 1880); *Asterropteryx modestus* Bleeker, 1875.

Type material SYNTYPES: 2, MNHN A-1568; 1, MNHN 2099.

Type locality. Saint Maurice River, Reunion Island.

Material examined. See list under "Specimens examined" above.

Differential diagnosis. *Hypseleotris cyprinoides* is distinguished from all other *Hypseleotris* by the presence of scales extending anteriorly to the interorbital region.

Description

Dorsal VI+I,8-9; anal I,9-11; vertebrae 14+10; dorsal pterygiophore formula 3-1221 or 3-12210. 24-27 scales in longitudinal series. Body laterally compressed, depth 3.9 to 4.6 in length. Eye diameter 3.7 to 4.6 in head, interorbital 1.0 to 1.2 times eye diameter. Head depressed, with small terminal mouth just reaching anterior margin of orbit. Caudal fin rounded.

Body covered with large scales, those on head and predorsal and preventral regions cycloid, the remainder ctenoid, small ctenoid scales on base of caudal, scales on top of head extend forward to between nostrils, operculum scaled, cheek scaled to beneath orbit. Colour in alcohol brown, countershaded darker on dorsal half, melanophores concentrated on posterior scale margins, sometimes coalesced into a dark midlateral stripe that may extend anteriorly onto operculum and snout. Distinct dark blotch on dorsal half of pectoral-fin base and lower half of caudal-fin base. Median fins mottled except second dorsal fin in males; second dorsal fin in males with pale spots on dark ground on proximal third to half, alternating dark and pale stripes on distal two thirds to half. This species is amply illustrated; examples include Allen (1991), Bruton (1996), Hoese (1986), and Kottelat *et al.* (1993).

Remarks

Hypseleotris cyprinoides is redescribed in order to clarify the identity of this widespread species, and to include the various taxa here synonymized with it. The nominal species *H. bipartita*, *H. dayi*, *H. tohizonae*, *H. leuciscus*, *H. modestus*, and *H. guentheri* all agree in meristic values and overall proportions, and have been distinguished on the basis of colouration. The colouration is somewhat variable, but there is always an obvious black spot present on the ventral half of the caudal peduncle at the edge of the caudal fin (in addition to the *Hypseleotris*-diagnostic blotch at the upper base of the pectoral fin). The remainder of the body may be faintly speckled with black (as described in the original description of *Eleotris* [*Hypseleotris*] *cyprinoides* Cuvier & Valenciennes 1837 [excerpted in Herre, 1927], and in accounts of *H. dayi* [Hoese, 1986]), or without any dark

pigment. Descriptions of *H. tohizonae* (Steindachner, 1880), *H. leuciscus* (Kottelat *et al.*, 1993), and *H. guentheri* (Günther, 1861; Bleeker, 1875; Ogilby, 1898; Allen, 1991) differ only in that the fine black spots are concentrated on the posterior margins of the scales, and aggregated into a lateral black band that extends to and meets the black spot on the caudal peduncle. In smaller individuals (up to approximately 35 mm SL) the body is more slender, without the lateral stripe and with clear or faintly brown-spotted median fins. Larger individuals feature the lateral stripe, a stockier body and median fins with more pronounced colouration: two pale stripes on black ground in the first dorsal fin, and three to four rows of pale spots on black ground on the proximal portion of the second dorsal fin. This degree of variation is also found in the other widespread *Hypseleotris* species, *H. compressa*, which exhibits variation in colour patterns and morphology that slowly changes with age, and may change rapidly depending upon mood or reproductive state (Larson & Hoese, 1996).

Australian *Hypseleotris*: Phylogeny

The remaining *Hypseleotris* species are endemic to Australia (and New Guinea, in the case of *H. compressa*), and form a monophyletic group with a decay index of two (Fig. 1, clade B). This group is diagnosed by the absence of preopercular and suborbital sensory pores (reversed in *H. compressa*, which has the pores); absence of interorbital scales, and elongated second dorsal fins with ten to thirteen elements (also reversed in *H. compressa*, which has the primitive condition of nine elements). Within the Australian clade, *H. klunzingeri* is the most basal; this species is widespread within southeastern Australia.

The remainder of the Australian species are divided in the phylogenetic hypothesis into two clades: the northwestern radiation (Fig. 1, clade C) is diagnosed by a reduction in caudal vertebrae (9-12 rather than 13-16; this state is also found in *Hypseleotris cyprinoides*). This clade contains the western Australian *H. aurea*, and a clade containing *H. compressa*, a coastal species with a wide distribution, ranging from Western Australia, around the northern and eastern shores of the continent, plus a radiation from the Kimberley region containing *H. ejuncida*, *H. kimberleyensis* and *H. regalis*, and *H. n.sp.* Katherine River (Northern Territory). Support for nodes, as seen in the decay indices, is low in this portion of the phylogeny, likely due to the lack of molecular data for the species *H. ejuncida*, *H. kimberleyensis*, and *H. regalis*. The Kimberley/Katherine River radiation is diagnosed by a reversal of the sexual dimorphism in head shape (these species lack the enlarged heads found in males of other *Hypseleotris* species) and by the presence of 13 precaudal vertebrae. *Hypseleotris aurea* also features 13 precaudal vertebrae, and a reversal of the other *Hypseleotris*-diagnostic sexual dimorphism character, the posterior elongation of the dorsal and anal fin-rays in males. The widespread salt-tolerant *H. compressa* is nested within the northwestern radiation, and is characterized by reversals of two characters, the presence of preopercular and suborbital head sensory canal pores, and a reduction of the number of elements in the second dorsal fin. This placement contradicts an earlier suggestion (Hoese & Allen, 1983) that *H. compressa* is the sister species to *H. cyprinoides*.

A second clade of Australian *Hypseleotris* (Fig. 1, clade D) occurs in southeastern Australia, and includes the species *H. galii*, and three undescribed species: *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's). Morphological patterns evident in this clade include a loss of the dark blotch in the ventral portion of the caudal fin, and increases in both the number of anal-fin elements and dorsal-fin spines; the insertion pattern of the dorsal spines also varies widely. These four species also share with *H. klunzingeri* a striped, brightly coloured pattern lacking spots in the second dorsal fin of males; other *Hypseleotris* species exhibit a pattern of pale spots in combination with striping.

Other phylogenetically informative characters may exist concerning the reproductive biology of *Hypseleotris*, but this character system was not included in the phylogeny, as the information was lacking for many species. Auty (1978) notes the eggs of *H. compressa* are more typical of marine pelagic eggs than freshwater demersal ones. The characteristics Auty (1978) lists include rapid development, thin chorion, poorly developed prolarvae, lacking a mouth and pigmented eye at hatching, high fecundity and presence of oil globules. Similar characteristics are also found in *H. klunzingeri* (Lake, 1967). We suspect these traits are ancestral within *Hypseleotris*. Another change in the reproductive biology occurred within clade D (Fig. 1); all these species have longer development times, well-developed larvae at hatching and lower fecundity (Anderson *et al.*, 1971; Unmack, 2000). Anderson *et al.* (1971) suggested this was an adaptation to the different environments inhabited by *H. klunzingeri* and *H. galii*, however, these species are sympatric in parts of their range, and the characteristics of *H. klunzingeri* appear to be ancestral.

A recent study of allozyme polymorphism among *Hypseleotris* from the lower Murray River [*H. klunzingeri*, *H. sp. 3* (Murray-Darling), *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's)] revealed a complex system of genetic interchange among species (Bertozzi *et al.*, 2000). These authors demonstrated the presence of three genetic types, HA, HB and HC, and found evidence of three hybrid classes designated HAxHB, HAxHX and HBxHX; HX is a type not sampled in pure form in their study. They provisionally identify HC as *H. klunzingeri*, the composite class HA, HB and HAxHB as representing the morphologically similar forms *H. sp. 3* (Murray-Darling) and *H. sp. 4* (Midgley's), and the HBxHX hybrid as *H. sp. 5* (Lake's). Our results support the contention that *H. sp. 5* (Lake's) hybridizes with sympatric *H. sp. 4* (Midgley's) from the Barcoo River; in the phylogeny, these *H. sp. 5* (Lake's) individuals are nested deep within the *H. sp. 4* (Midgley's) clade (Fig. 1). In this case, it is possible the *H. sp. 5* (Lake's) individuals clustered within *H. sp. 4* (Midgley's) are hybrids with *H. sp. 4* (Midgley's) mothers, or simply that a mitochondrial introgression has taken place between the two species. The molecular marker used in this study is mitochondrial, therefore, phylogenetic analysis of this gene reveals only the maternal lineage patterns. Two other possibilities exist that could explain the phylogenetic pattern observed among species of *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's); the species are not distinct, or the specimens used are improperly identified. These possibilities are difficult to evaluate because the species have not been formerly described, however, all specimens used in the molecular analysis were identified using the characters described in Larson & Hoese

(1996) and Allen (1989); these characters provide a morphological basis for distinguishing the two taxa. Additional molecular studies are underway, using both mitochondrial and nuclear markers, that will address questions of identity and distinction between *H. sp. 4* (Midgley's) and *H. sp. 5* (Lake's).

Australian *Hypseleotris*: Biogeography

Overall, a historical geographic pattern of southward expansion from Indo-Pacific Islands to Australia is indicated in the phylogeny of *Hypseleotris* species. Our phylogenetic hypothesis is consistent with an origin in Southeast Asia. An ancestor to *H. cyprinoides* may have dispersed via marine environments from South East Asia and became widespread, eventually reaching southeastern Australia where it invaded freshwaters. The Australian fish fauna is relatively depauperate and lacks the dominant ostariophysan groups found throughout most of the range of *Hypseleotris* (Allen, 1989). This situation may have provided an opportunity for *Hypseleotris* to radiate within Australia once it invaded freshwater. Unfortunately, no fossil evidence exists that could indicate when this invasion occurred.

The phylogeny indicates the presence of two radiations, one in northwestern Australia (Fig. 1, clade C), and a second within southeastern Australia (Fig. 1, clade D). It is unclear why radiations occurred in these different climatic regions, or why no *Hypseleotris* other than *H. compressa* occur in the geographic areas in between, nor in southern New Guinea where seemingly suitable habitats exist. One possible explanation is that invasion of the western coast was via central Australian drainages, thus invasion of the northern coastal drainages simply never occurred. The Central Australian Province (which contains the basal Australian species *H. klunzingeri*) shares a drainage divide with the Paleo-Victoria drainage (as well as many other drainages) that may have allowed aquatic fauna to move (Unmack, 2001) prior to the onset of aridity that has gradually increased since mid-Miocene (Van de Graaff *et al.*, 1977). From the Victoria River, movement to other drainages would have been relatively easy between adjacent rivers during lowered sea levels or over single drainage divides into rivers draining the Pilbara region (Unmack, 2001). Most *Hypseleotris* species within northwestern Australia are not widespread, suggesting suitable habitats are limited, or their migratory abilities are poor (which does not seem to be the case in other *Hypseleotris* species). The Kimberley and Pilbara regions have high proportions of endemic species, many of which have limited ranges (Unmack, 2001). It is possible these species are remnants persisting in small refugia within the numerous gorges in these regions that provide a more permanent water supply.

Southeastern Australia contains an assortment of *Hypseleotris* species with complex biogeographic relationships. The primary biogeographic barrier within this region is the Eastern Highlands, the mountain range that separates the Eastern Province from the Murray-Darling Province. A smaller mountain range separates the Murray-Darling Province from the Central Australian Province (including the Lake Eyre Basin). Three of the species are found in more than one of these provinces, and it is likely they have had opportunities to move at different times between provinces. A number of closely related fish species

pairs or conspecifics are known to occur between Eastern Province and Murray-Darling Province (c. 20 pairs or conspecifics total) and Murray-Darling Province and Central Australian Province (c. 13 pairs or conspecifics total), suggesting faunal exchange has been common (Unmack, 2001). Previously, Unmack (2000) suggested qualitative morphological differences existed between Eastern Province and Murray-Darling Province/Central Australian Province populations of *H. klunzingeri* and *H. sp. 4* (Midgley's). Our results are broadly compatible with that hypothesis, and further studies are underway examining the detailed intraspecific phylogenies of southeastern Australian freshwater fish species, based on both nuclear and mitochondrial markers.

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