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## *Nannoperca pygmaea*, a new species of pygmy perch (Teleostei: Percichthyidae) from Western Australia

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### Abstract

A new species of pygmy perch (Percichthyidae) from south-western Australia is described on the basis of 15 specimens collected from the Hay River system. *Nannoperca pygmaea* **sp. nov.** differs from the sympatric congener *N. vittata* (Castelnau) by the absence of dark pigment on the ventral surface anterior to the anus, the possession of thin latero-ventral stripes, generally fewer dorsal rays and fewer anal rays, hind margin of scales on caudal peduncle without distinct pigment, and a more pronounced spot (ocellus) that is surrounded by a halo at the termination of the caudal peduncle. The new species is distinguished from congeners *Nannoperca australis* Günther, *N. oxleyana* Whitley and *N. variegata* Kuitert and Allen in possessing an exposed and serrated preorbital bone and jaws that may just reach to below the anterior margin of the eye, versus a smooth and hidden preorbital and the jaws reaching to at least below the pupil; and from the remaining congener, *N. obscura* (Klunzinger) in possessing a distinct haloed ocellus at base of caudal fin versus an indistinct barring, as well as a dark spot behind operculum, and the lack of dusky scale margins. It differs from the other sympatric pygmy perch found in the region, *N. balstoni* Regan, by the presence of an exposed rear edge of the preorbital (vs. hidden under skin), fewer transverse scale rows (13 vs. 15–16), small mouth (rarely reaching eye vs. reaching well beyond eye), ctenoid (vs. cycloid) body scales, generally fewer pectoral rays and smaller maximum size. Allozyme analyses unequivocally demonstrate that sympatric populations of *N. pygmaea* **sp. nov.** and *N. vittata* belong in different genetic lineages, display no genetic intermediates, and are diagnosable by fixed allozyme differences at 15 different loci. Due to its extremely restricted range, where it is known from only 0.06 km<sup>2</sup>, *N. pygmaea* **sp. nov.** requires urgent legislative protection.

**Key words:** sympatric species, *Nannoperca vittata*, *Nannatherina balstoni*, Hay River, Mitchell River, South West Coast Drainage Division, endemic fishes

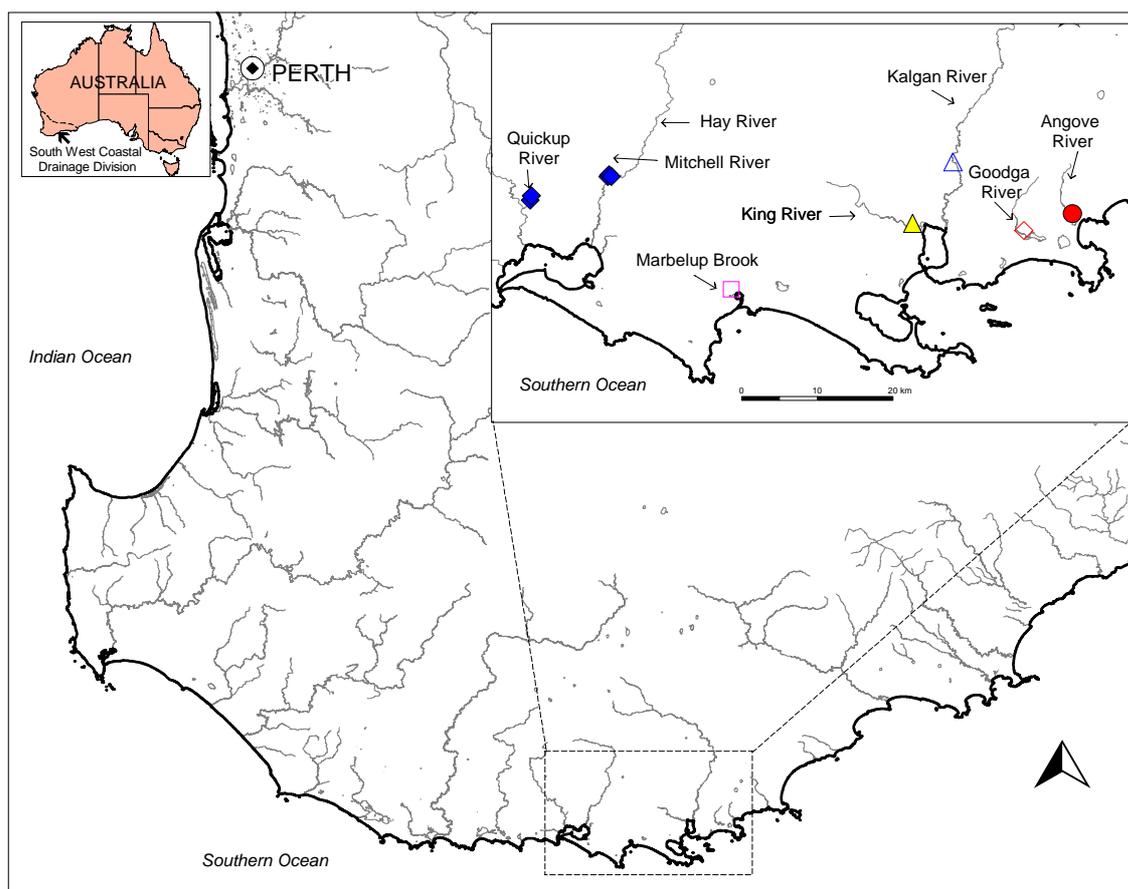
### Introduction

The pygmy perches, *Nannoperca* and *Nannatherina*, are represented by six species that are restricted to southern Australia and are placed either within the Nannopercidae (e.g. Allen 1989, Kuitert *et al.* 1996, Allen *et al.* 2002) or Percichthyidae (e.g. Kuitert & Allen 1986, Jerry *et al.* 2001, Paxton *et al.* 2006, Unmack *et al.* 2011). Jerry *et al.* (2001) demonstrated that the pygmy perches are monophyletic with *Macquaria* and placed them within the Percichthyidae. Jerry *et al.* (2001) and Kuitert *et al.* (1996) suggest that the pygmy perch genus *Edelia* should be incorporated with *Nannoperca*, based on molecular genetic criteria and reflecting minor anatomical differences, such as the posterior margin of the preorbital bone being either hidden by skin (*Edelia*) or exposed (*Nannoperca*), however Allen *et al.* (2002) and Paxton *et al.* (2006) retain *Edelia*. Jerry *et al.* (2001), based on 12S rRNA, found no basis for recognising *Edelia*, with *E. vittata* and *E. obscura* being unmistakably sister taxa to *Nannoperca australis*, *N. oxleyana* and *N. variegata*. Unmack *et al.* (2011) in their phylogenetic revision of the pygmy perches support the use of *Nannoperca* for all species of pygmy perch except *Nannatherina balstoni*.

We accept that there are currently three described endemic species of percichthyid in south-western Australia belonging to three genera, *Nannoperca*, *Nannatherina* and *Bostockia* (Fig. 2b, c, d) (Morgan *et al.* 1998, Jerry *et al.*

2001, Allen *et al.* 2002, Unmack *et al.* 2011). This region encompasses the South West Coastal Drainage Division (Fig. 1), and contains the highest proportion of endemic freshwater fishes of all of Australia's major drainage divisions (Morgan *et al.* 1998, Allen *et al.* 2002). Eight of the 10 freshwater fish species are endemic, including all three species of percichthyid, *Nannoperca vittata*, *N. balstoni* and *Bostockia porosa*. *Nannatherina balstoni* is extremely rare throughout its range (Morgan *et al.* 1995, 1998, Morgan 2009) and is listed in Australia under the Environmental Protection and Biodiversity Conservation Act 1999 as vulnerable (Morgan 2009). The remaining two percichthyids are much more widespread but have undergone considerable range reductions due to loss of habitat, secondary salinisation of habitats and competition with introduced fishes (Morgan *et al.* 1998, 2003, 2004, Beatty *et al.* 2011).

Unmack *et al.* (2011) demonstrated that *N. vittata* shows extensive genetic heterogeneity across its range, and suggest that this is indicative of there being multiple species at the catchment scale. Sampling of fishes in the Hay River catchment (Fig. 1) during 2009 revealed three morphologically and phenotypically distinctive forms of pygmy perch (Fig. 2). This included two described species, *N. vittata* and *N. balstoni*, and a further form that appeared to be a distinct yet undescribed species. The latter fish most closely resembled *N. vittata*, having a small mouth that rarely reaches beneath the anterior margin of the eye (although the maxilla may just reach it), whereas *N. balstoni* has a mouth that extends beyond the anterior margin of the eye, the maxilla reaching to beneath the middle of the eye. While morphological characters have historically been used to assess the taxonomic status of phenotypically distinctive forms, it is now common practice to use a combination of both morphological and molecular genetic characters for this purpose. We therefore include both genetic and morphological data in the description of this newly discovered pygmy perch from south-western Australia.



**FIGURE 1.** Sampling sites, in the South West Coastal Drainage Division, Western Australia. Both sites 1 and 2 in the Mitchell River and in the Quickup River are in close proximity to each other.

## Materials and methods

**Capture sites and morphological and meristic counts.** A total of 20 sites was sampled throughout the Hay River catchment (Fig. 1), with the undescribed species of pygmy perch only captured at three adjacent sites including: (1) the lower section of the Mitchell River ~300 m upstream of the confluence with the Hay River (34°53.15'S, 117°29.27'E), (2) in the Hay River at the confluence of the Mitchell River (34°53.27'S, 117°29.14'E), and (3) in the Hay River (34°53.28'S, 117°29.45'E) ~500 m upstream of site 2. Using the methodology for morphometric measurements and meristic counts as given in Kuitert and Allen's (1986) synopsis of the Australian pygmy perches which includes the original description of *Nannoperca variegata*, a total of 15 individuals was assessed and compared to 22 sympatric *N. vittata* from these sites. Comparisons were then made between the undescribed species and relevant published information including that detailed in Kuitert and Allen (1986), Allen (1989), Kuitert *et al.* (1996) and Allen *et al.* (2002). Type specimens have been deposited in the Western Australian Museum (WAM) and the South Australian Museum (SAM).

**Allozyme procedures.** Muscle tissues were collected from 13 individuals representing the two morphotypic forms found in the Hay River catchment. These tissues were immediately frozen on site in liquid nitrogen and later stored at -70°C until required for allozyme analysis. Reference tissues representing all other populations of *N. vittata* from rivers including and to the east of the Hay River catchment (n = 28) were obtained from the Australian Biological Tissue Collection, housed at the SAM.

Allozyme electrophoresis of muscle homogenates was undertaken on cellulose acetate gels ('Cellogel', M.A.L.T.A., Milan) according to the principles and procedures detailed in Richardson *et al.* (1986). The following enzymes or non-enzymatic proteins displayed bands of sufficient activity and resolution to allow allozymic interpretation:- ACON, ACP, ACYC, ADA, ADH, AK, ALD, ALDH, AP, CA, CK, ENOL, EST, FDP, FUM, GAPD, GLO, GOT, GP, G6PD, GPI, GSR, IDH, LAP, LDH, MDH, ME, MPI, NDPK, PEP-A, PEP-B, PEP-D, PGAM, 6PGD, PGK, PGM, PK, and TPI. Enzyme and locus abbreviations, electrophoretic conditions and stain recipes are described elsewhere (Richardson *et al.* 1986, Hammer *et al.* 2007). Allozymes were designated alphabetically and multiple loci, where present, were designated numerically, both in order of increasing electrophoretic mobility.

As discussed in detail elsewhere (Horner and Adams 2007), the assessment of species boundaries using allozyme data is best undertaken by initially adopting a multivariate analytic procedure such as Principal Coordinates Analysis (PCA) that employs individuals rather than populations or sites as the unit of analysis. Unlike site-based assessments, PCA allows both within-site and between-site heterogeneity to be concurrently assessed, and also facilitates the identification of hybrids and of individuals bearing the genetic signature of introgression. An initial PCA was undertaken on a pairwise matrix of Rogers' genetic distance among individuals, following the methodology presented in Hammer *et al.* (2007). Thereafter, a scatterplot of PCA scores in the first two dimensions was assessed for the presence of discrete clusters of individuals. The raw genotypes were then examined to determine if any of these genetic lineages were diagnosable by fixed differences, as would be expected where two clusters represent different biological (sympatric) or evolutionary (allopatric) species. Relationships among the genetic lineages identified in the PCA were examined by constructing an unrooted Neighbor Joining (NJ) tree based on a pairwise matrix of Nei's unbiased genetic distances among sites, following the methodologies presented in Hammer *et al.* (2007).

## Results

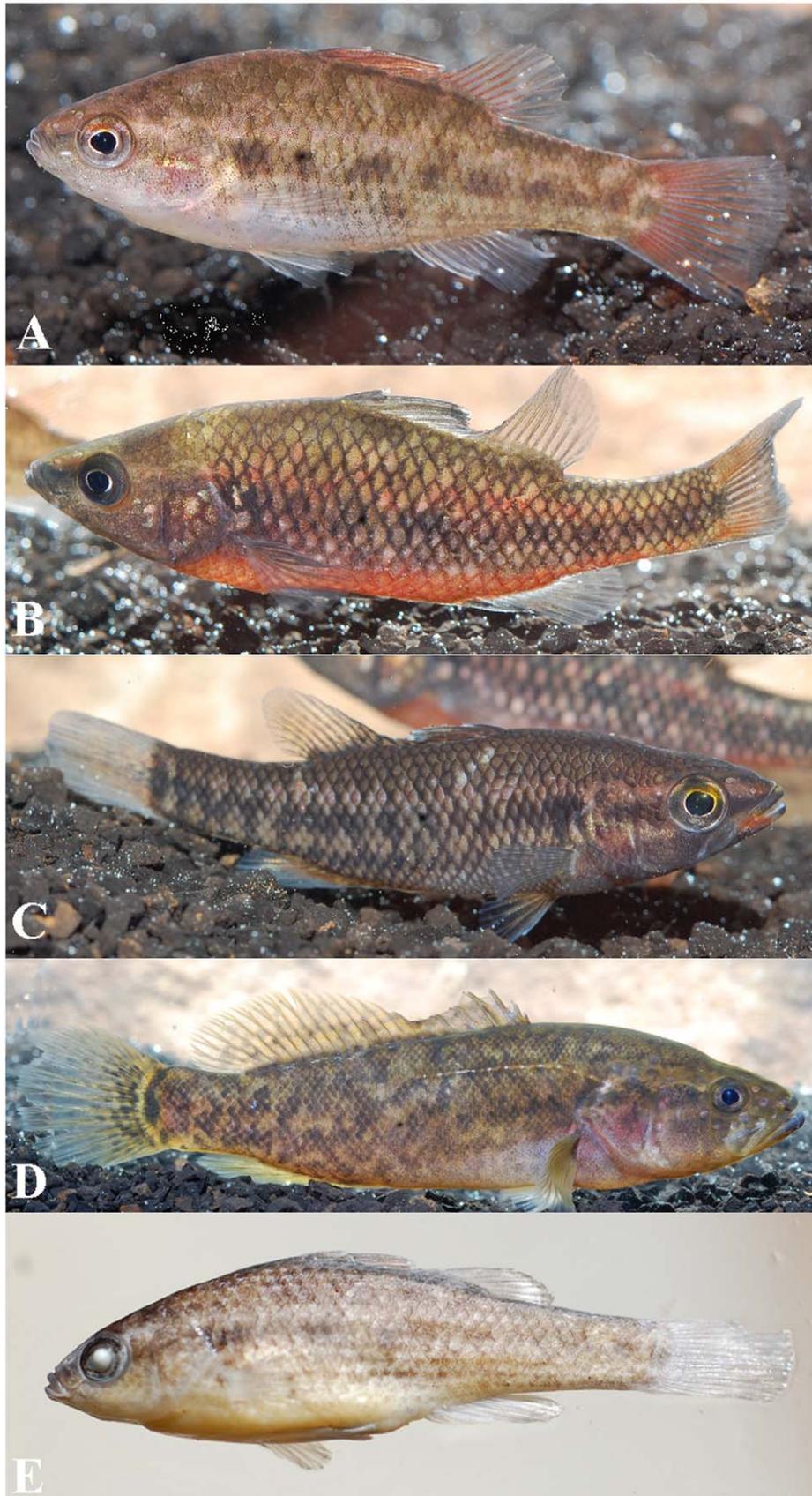
### Systematics

#### *Nannoperca pygmaea* sp. nov.

Little Pygmy Perch

Fig. 2A

**Holotype.** WAM P.33379-001, 31.6 mm SL, Hay River, Western Australia (34°53.28' S, 117°29.45' E), collected by D.L. Morgan and S.J. Beatty, 9 September 2009 (Fig. 2).



**FIGURE 2.** Percichthyidae of the South West Coastal Drainage Division, Western Australia. A. *Nannoperca pygmaea* **sp. nov.** (WAM P.33380–001, 31.8 mm SL). B. *Nannoperca vittata*. C. *Nannatherina balstoni*. D. *Bostockia porosa*. Bottom, holotype *N. pygmaea* **sp. nov.** Photographs S. Beatty and D. Morgan.

**Paratypes.** WAM P.33380–001, 10 specimens, 26.8–38.0 mm SL, Mitchell River, Western Australia (34°53.15' S, 117°29.27' E), collected by D.L. Morgan and S.J. Beatty, 28 October 2009; WAM P.33379.02, 1 specimen, 33.2 mm SL, Hay River/Mitchell River confluence, Western Australia (34°53.27' S, 117°29.14' E), collected by D.L. Morgan and S.J. Beatty, 9 September 2009; SAM FISHY6: Esp.09001, Esp.09003, Esp.09004 (Fig. 2), 3 specimens, 28.6–38.7 mm SL, Mitchell River and Hay River confluence, Western Australia (34°53.27' S, 117°29.14' E), collected by D.L. Morgan and S.J. Beatty, 9 December 2009.

**Diagnosis.** A species of *Nannoperca* in having a small mouth, a deeply notched dorsal fin, a poorly developed two-part and interrupted lateral line. It is distinguished from the congeneric *N. australis*, *N. oxleyana* and *N. variegata* in possessing an exposed and serrated preorbital bone and the jaws may just reach to below the anterior margin of the eye, versus possessing a smooth and hidden preorbital and the jaws reaching to at least the pupil. It is distinguished from *N. vittata* by: the possession of 5–10 thin lateral stripes most obvious below lateral line; poorly developed tube scales versus well developed in *N. vittata*; a more distinct haloed blackish spot resembling an ocellus at the base of the caudal fin; the hind margin of the scales on the caudal peduncle are without distinct pigment as in *N. vittata*; and the belly is without a colour pattern. It is distinguished from *N. obscura* in possessing a distinct ocellus at the base of caudal fin versus an indistinct barring, as well as a dark spot behind operculum and lack of dusky scale margins. It is distinguished from the other sympatric pygmy-perch species in the region, *N. balstoni*, in possessing an exposed rear edge of the preorbital (compared to being hidden under the skin in *N. balstoni*), has fewer transverse scale rows (13 vs. 15–16), a smaller mouth (rarely reaching eye vs. reaching well beyond the eye), ctenoid body scales (vs. cycloid), generally fewer pectoral rays and a smaller maximum size.

**Description.** Dorsal-fin rays holotype VIII, 8 (paratypes VII–IX, 8 or 9); anal-fin rays III, 6 (paratypes III, 6 or 7); pectoral-fin rays 11 (paratypes 10 or 11); body scales ctenoid; lateral-line scales with marginally developed tubes; lateral line scales 18+13; horizontal scale rows at level of anal fin origin 13–15; gill rakers on first arch 3+7 (2–3 + 5–7); jaw just reaching to below anterior part of eye. Body relatively slender, laterally compressed, greatest body depth 3.0 (2.7–3.4) in SL. Head relatively short with pointed snout, its length 3.3 (2.9–3.6) in SL. Following proportions are in head length: snout length 4.7 (3.9–6.2), exposed maxilla length 4.9 (4.3–6.2), eye width 3.5 (3.3–3.9), interorbital width 3.6 (3.1–4.0), caudal peduncle depth 1.7 (1.5–2.1), caudal peduncle length 1.2 (1.1–1.4), caudal fin length 1.3 (1.2–1.6), pectoral fin length 1.7 (1.4–2.1), pelvic fin length 1.7 (1.5–1.9), first dorsal spine length 3.3 (2.8–4.2), first anal spine length 5.5 (4.7–8.0), second anal spine length 2.7 (2.3–3.1), third anal spine length 3.0 (2.5–3.9) (Tables 1, 2 and 3).

Longest dorsal spine 2nd, longest soft dorsal ray 3rd or 4th, longest soft anal ray 2nd or 3rd, pelvic and pectoral fins usually equal in length. Reduced blackish spot behind edge of gill cover, coloration on ventral surface anterior to anus limited to one or two dark melanophores, a distinct ocellus at base of caudal fin, fins often orange, large brownish dorso-lateral blotches often merging, series of brownish mid-lateral blotches commencing behind operculum, terminating on caudal peduncle; hind margin of scales on caudal peduncle without distinct pigment; two spines on hind margin of operculum, almost equal; 5–10 thin lateral stripes most obvious below lateral line, tube scales poorly developed.

**Coloration in preservative.** Generally pale fins and lateral and dorso-lateral blotches light to dark brown in colour except for ventral surface, which appears opaque, thin ventro-lateral stripes more prominent than in live specimens, opercular spot distinct, haloed ocellus on caudal peduncle at base of caudal fin very distinct.

**Etymology.** The specific epithet *pygmaea* is the feminised form of the Latin noun *pygmaeus* meaning “dwarf” applied as a noun in apposition, and in reference to this being the smallest of the pygmy perches. Common name Little Pygmy Perch applied in recognition of the relative small size.

**Genetic assessment.** The final allozyme dataset comprised genotypes at 56 putative loci for 41 fish from nine sites, and included sympatric series of both *N. pygmaea* (n = 8) and *N. vittata* (n = 8) from the Hay River catchment. An initial PCA (Fig. 3) clearly demonstrated the presence of two major genetic lineages, diagnosable by a complete absence of shared alleles at 15 different allozyme loci (Table 4). The absence of heterozygous individuals at any of the 15 diagnostic loci demonstrates that these two lineages do not interbreed in sympatry and display no evidence of introgression in allopatry, i.e. the unequivocal genetic signature of different biological species. The NJ tree among populations (Fig. 4) further demonstrates their genetic distinctiveness. The two species display a mean Nei D of 0.34, considerably larger than the maximum between-population value of 0.06 within *N. vittata*.

**TABLE 1.** Mean and range (in parentheses) of proportional external measurements of *Nannoperca vittata* and type specimens of *Nannoperca pygmaea* **sp. nov.** from the Mitchell/Hay River catchment, expressed as a percentage of standard length (SL).

| Species                      | <i>Nannoperca vittata</i> |  | <i>Nannoperca pygmaea</i> |                      |
|------------------------------|---------------------------|--|---------------------------|----------------------|
|                              | n = 22                    |  | Holotype                  | Paratypes n = 14     |
| mean (range) SL (mm)         | 40.02 (28.1–50.7)         |  | 31.6                      | 32.38 (28.6–38.7)    |
| Total length (TL)            | 121.58 (115.58–127.01)    |  | 124.7                     | 123.69 (116.4–128.3) |
| Greatest body depth          | 33.97 (30.29–39.59)       |  | 29.7                      | 33.2 (29.1–36.5)     |
| Head length                  | 31.19 (27.45–33.78)       |  | 28.3                      | 30.8 (28.2–34.3)     |
| Snout length                 | 6.64 (5.80–8.09)          |  | 5.8                       | 6.8 (5.0–9.3)        |
| Maxilla length               | 7.11 (5.36–8.42)          |  | 5.9                       | 6.3 (4.8–7.3)        |
| Eye diameter                 | 8.02 (6.98–9.16)          |  | 7.5                       | 8.7 (7.8–10.1)       |
| Caudal peduncle length       | 22.85 (20.73–27.56)       |  | 25.9                      | 24.8 (21.6–26.6)     |
| Caudal peduncle depth        | 16.89 (14.28–21.78)       |  | 18.4                      | 17.9 (15.6–19.8)     |
| Pectoral fin length          | 18.30 (15.57–22.22)       |  | 17.5                      | 18.5 (15.0–22.0)     |
| Pelvic fin length            | 18.48 (16.84–20.44)       |  | 17.5                      | 18.3 (16.3–20.2)     |
| Length of dorsal fin base    | 34.70 (32.30–36.19)       |  | 33.0                      | 33.6 (29.2–39.0)     |
| Length of anal fin base      | 15.43 (13.30–17.51)       |  | 12.7                      | 14.9 (11.9–17.9)     |
| Caudal fin length            | 22.23 (20.06–27.65)       |  | 23.1                      | 23.3 (20.2–25.2)     |
| 1st dorsal spine length      | 9.29 (7.26–12.15)         |  | 7.6                       | 9.5 (7.5–11.8)       |
| 2nd dorsal spine length      | 19.25 (16.80–21.85)       |  | 17.9                      | 18.9 (16.3–21.1)     |
| Longest dorsal spine length  | 2nd                       |  | 2nd                       | 2nd                  |
| Longest soft dorsal ray      | 3rd                       |  | 3rd                       | 3rd or 4th           |
| 1st anal spine length        | 5.93 (4.88–7.11)          |  | 5.2                       | 5.7 (3.7–6.2)        |
| 2nd anal spine length        | 11.42 (9.27–13.36)        |  | 10.4                      | 11.6 (10.1–13.5)     |
| 3rd anal spine length        | 9.76 (7.99–11.24)         |  | 9.4                       | 10.2 (7.9–11.5)      |
| Longest soft anal ray length | 3rd or 4th                |  | 3rd                       | 2nd or 3rd           |

**TABLE 2.** Dorsal and anal fin-ray counts for *Nannoperca* spp. and *Nannatherina balstoni* (*N. vittata* and *N. pygmaea* from this study, others and *N. vittata* from Kuiter and Allen (1986)).

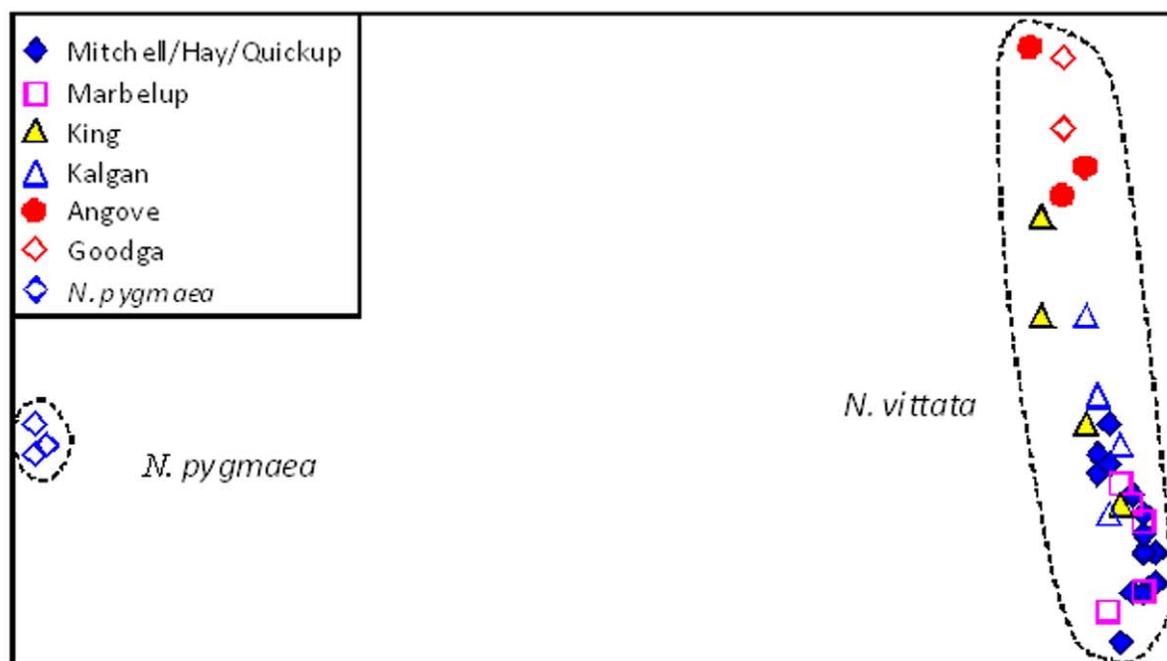
|                     | Dorsal spines |     |      |    | Soft dorsal rays |    |    |    |    | Soft anal rays |    |    |    |    |
|---------------------|---------------|-----|------|----|------------------|----|----|----|----|----------------|----|----|----|----|
|                     | VI            | VII | VIII | IX | 7                | 8  | 9  | 10 | 11 | 6              | 7  | 8  | 9  | 10 |
| <i>N. pygmaea</i>   |               | 1   | 6    | 8  |                  | 10 | 5  |    |    | 5              | 10 |    |    |    |
| <i>N. australis</i> | 1             | 14  | 59   | 2  | 1                | 19 | 48 | 8  |    | 2              | 38 | 35 | 1  |    |
| <i>N. obscura</i>   |               |     | 8    | 24 | 3                | 25 | 4  |    |    | 7              | 24 | 1  |    |    |
| <i>N. oxyleyana</i> | 2             | 27  | 1    |    | 1                | 25 | 4  |    |    |                | 25 | 5  |    |    |
| <i>N. variegata</i> |               |     | 19   | 2  |                  |    | 14 | 7  |    |                |    | 10 | 11 |    |
| <i>N. vittata</i>   |               | 3   | 38   | 2  |                  | 6  | 26 | 10 | 1  |                | 4  | 36 | 3  |    |
| <i>N. balstoni</i>  |               | 2   | 16   | 2  |                  |    | 1  | 7  | 12 |                |    | 1  | 15 | 4  |

**Discussion.** *Nannoperca pygmaea* represents the third species of pygmy perch (and the fourth percichthyid) discovered in Western Australia. The number of endemic freshwater fishes of Australia's South West Coast Drainage Division is now nine and the proportion of endemic species in the region now stands at 82%; the highest of any Australian drainage division. A recent molecular overview of pygmy perches (Unmack *et al.* 2011) revealed evidence for two very distinct genetic lineages among a handful of *N. vittata* populations further to the west and north of the Hay River catchment. Unmack *et al.* (2011), based on genetic evidence, suggest that one of the two lineages warrants elevation to species status pending morphological diagnosis. Thus it is likely that there are at

least four species of pygmy perch in this region, long recognised as one of the planet’s hotspots for biodiversity and endemism (Myers *et al.* 2000).

**TABLE 3.** Pectoral-fin ray and gill raker (lower limb of first arch) counts for *Nannoperca* spp. and *Nannatherina balstoni* (*N. vittata* and *N. pygmaea* from this study, others and *N. vittata* from Kuitert and Allen (1986)).

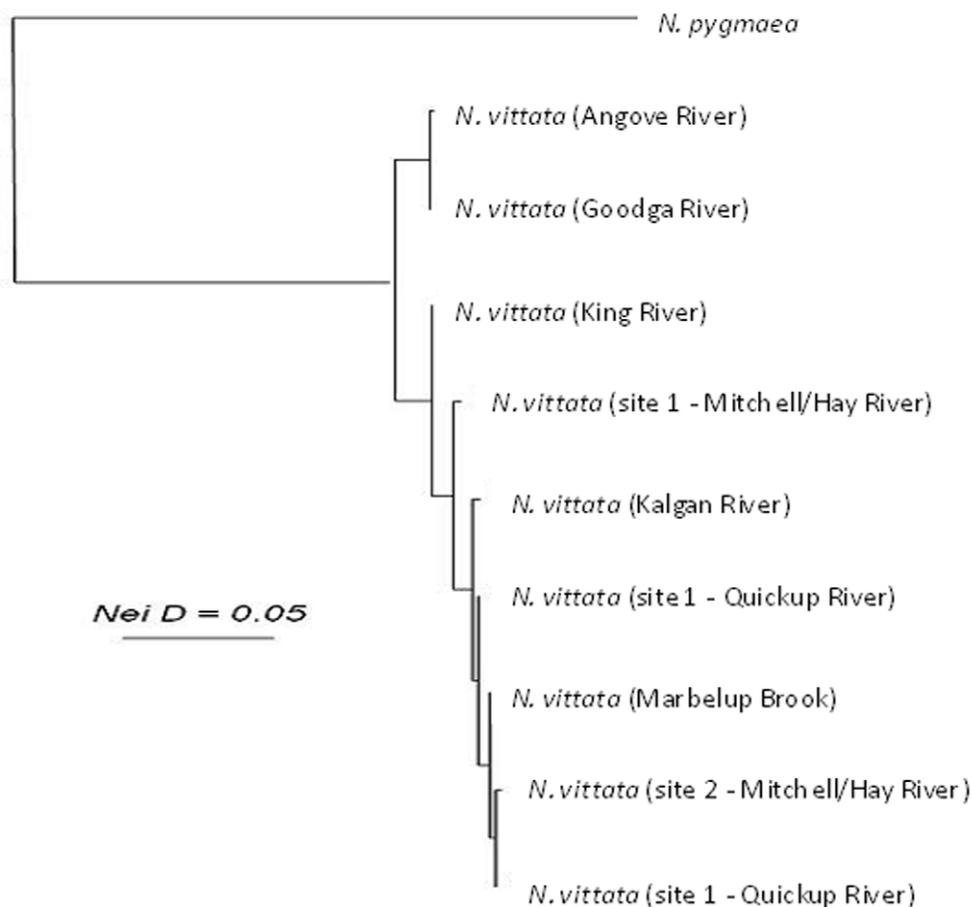
|                     | Pectoral rays |    |    |    |    |    | Gill rakers |   |    |   |    |    |   |    |
|---------------------|---------------|----|----|----|----|----|-------------|---|----|---|----|----|---|----|
|                     | 10            | 11 | 12 | 13 | 14 | 15 | 3           | 4 | 5  | 6 | 7  | 8  | 9 | 10 |
| <i>N. pygmaea</i>   | 3             | 12 |    |    |    |    |             |   |    | 1 | 1  | 9  | 1 |    |
| <i>N. australis</i> |               | 4  | 40 | 30 | 2  |    |             |   |    | 5 | 23 | 14 | 7 |    |
| <i>N. obscura</i>   |               | 2  | 23 | 6  |    |    |             |   |    |   | 4  | 12 | 4 |    |
| <i>N. oxyleyana</i> |               | 1  | 22 | 9  |    |    |             |   |    | 6 | 8  | 18 |   |    |
| <i>N. variegata</i> |               |    |    |    | 15 | 6  | 3           | 5 | 13 |   |    |    |   |    |
| <i>N. vittata</i>   | 2             | 10 | 18 | 10 | 1  |    |             |   |    | 5 | 22 | 9  |   |    |
| <i>N. balstoni</i>  |               |    |    | 13 | 7  |    | 2           | 2 | 11 | 3 | 2  |    |   |    |



**FIGURE 3.** Principal Co-ordinates Analysis (PCA) of *Nannoperca vittata* from six river systems (Mitchell (Hay) River, Marbelup Brook, King River, Kalgan River, Angove River, Goodga River) and *Nannoperca pygmaea* genotyped in the allozyme study. The relative PCA scores have been plotted for the first (X-axis) and second (Y-axis) dimensions, which individually explained 65% and 9%, respectively, of the total multivariate variation. See Fig. 1 for site localities.

**Habitat and conservation considerations.** Explanation of how *N. pygmaea* evaded discovery for so long lies in the fact that its habitats are only seasonally accessible by vehicle and it has an extremely narrow range. In terms of distribution, *N. pygmaea* is the most restricted species of pygmy perch, currently known from a geographical area of only 0.06 km<sup>2</sup>, and a stream length of 1.2 km. It is one of Australia’s most geographically restricted freshwater fish species. Its habitats are tannin stained, acidic and shallow *Melaleuca raphiophylla* streams (Fig. 5). Flow is seasonal, characteristic of this ‘Mediterranean Climatic’ region, with the site on the Mitchell River drying out each summer. Although the Mitchell River supports intact natural vegetation, much of the Hay River catchment is cleared of its natural vegetation (52%). The pools on the Hay River are therefore critical summer

refuges for the species, although the catchment is becoming increasingly affected by secondary salinisation (Mayer *et al.* 2005). For example, between 1993 and 2002 the mean salinity of the Hay River was 2300 mg.L<sup>-1</sup>, which was an overall increase of 400 mg.L<sup>-1</sup> from the previous decade (Mayer *et al.* 2005). Although mean conductivity in the Mitchell River generally remains below 0.5 mS.cm<sup>-1</sup>, the type locality in the Hay River increases in mean conductivity from <2.9 mS.cm<sup>-1</sup> in winter and early spring, to almost 11 mS.cm<sup>-1</sup> in late summer as these habitats decrease in size to small isolated pools. Stream secondary salinisation is a recognised threat to south-western Australia's freshwater fishes (Morgan *et al.* 2003), and while the salinity tolerance of *N. pygmaea* is unknown, it is likely to be similar to the sympatric pygmy perches *N. balstoni* and *N. vittata*, which have acute tolerances of 8.2 and 14.6 g.L<sup>-1</sup>, respectively (Beatty *et al.* 2011). Mean winter temperatures are ~12°C, increasing to over 23°C in summer. As a consequence of the rarity and restricted geographic range of *N. pygmaea*, the species requires urgent protection at the state and federal level.



**FIGURE 4.** NJ tree based on pairwise Nei Ds among sties for *Nannoperca vittata* in seven catchments (Angove River, Goodga River, King River, Mitchell/Hay River, Kalgan River, Quickup River and Marbelup Brook) and *Nannoperca pygmaea* in the Mitchell/Hay River. See Fig. 1 for site localities.

**TABLE 4.** Allele frequencies for all variable loci at the sites sampled for *Nannoperca vittata* and *N. pygmaea*. Frequencies of all but the rarer/rarest alleles are expressed as percentages and shown as superscripts (allowing the frequency of each rare allele to be calculated by subtraction from 100%). The allozyme loci, which diagnose *N. pygmaea* from *N. vittata*, are identified with an asterisk. *Nannoperca vittata* sites are referred to by the following abbreviations: AN = Angove R., GO = Goodga R., KA = Kalgan R., KI = King R., QU#1 and QU#2 = Quickup R., MH = Mitchell and Hay R. (*N. pygmaea* is sympatric with *N. vittata* at site MH#2). See Fig. 1 for site localities. Invariant loci: *Acp*, *Ald1*, *Ap*, *Ca1*, *Ck*, *Eno11*, *Gapd1*, *Gapd2*, *Glo*, *Gp1*, *Gp2*, *Gsr*, *Idh*, *Ldh1*, *Mdh1*, *Mpi*, *Ndpk*, *Pgam*, *Pgk*, *Pgm*, *Pk1*, *Pk2*, and *Tpi1*.

| Locus           | AN<br>(3)          | GO<br>(3)          | KA<br>(4)          | KI<br>(4)                           | MA<br>(5)                           | QU#1<br>(3)                         | QU#2<br>(3)  | MH#1<br>(3)                         | MH#2<br>(5)                         | <i>N. pygmaea</i><br>(8) |
|-----------------|--------------------|--------------------|--------------------|-------------------------------------|-------------------------------------|-------------------------------------|--|-------------------------------------|-------------------------------------|--------------------------|
| <i>Acon1</i> *  | b                  | b                  | b <sup>75</sup> ,a | b <sup>75</sup> ,a                  | b <sup>70</sup> ,d                  | b                                   | b  | b                                   | b <sup>90</sup> ,a                  | c                        |
| <i>Acon2</i>    | b                  | b                  | b                  | b                                   | b                                   | b                                   | b  | b <sup>83</sup> ,a                  | b                                   | b                        |
| <i>Acon3</i>    | a                  | a                  | b <sup>62</sup> ,a | a <sup>88</sup> ,b                  | b <sup>70</sup> ,a                  | b <sup>50</sup> ,a <sup>33</sup> ,c | a <sup>50</sup> ,b                                   | a                                   | b                                   | a                        |
| <i>Acyc</i>     | b                  | b                  | b                  | b                                   | b                                   | b <sup>83</sup> ,a                  | b  | b                                   | b                                   | b                        |
| <i>Ada</i> *    | a                  | a                  | a                  | a                                   | a                                   | a                                   | a  | a                                   | a                                   | b                        |
| <i>Adh1</i>     | a                  | a                  | a                  | a                                   | a                                   | a <sup>67</sup> ,c                  | a <sup>83</sup> ,c                                   | a                                   | a <sup>90</sup> ,b                  | a                        |
| <i>Adh2</i>     | b                  | b                  | b                  | b                                   | b                                   | b                                   | b <sup>83</sup> ,a                                   | b                                   | b                                   | b                        |
| <i>Ak</i>       | b                  | b                  | b                  | b                                   | b                                   | b                                   | b  | b <sup>83</sup> ,a                  | b                                   | b                        |
| <i>Ald2</i>     | b <sup>83</sup> ,a | b <sup>83</sup> ,a | b                  | b                                   | b                                   | b                                   | b  | b                                   | b                                   | b                        |
| <i>Aldh</i>     | b                  | b                  | b                  | b                                   | b                                   | b                                   | b  | b                                   | b <sup>90</sup> ,a                  | b                        |
| <i>Ca2</i> *    | a                  | a                  | a                  | b <sup>75</sup> ,a                  | a <sup>80</sup> ,b                  | a                                   | a  | b <sup>67</sup> ,a                  | a <sup>63</sup> ,b                  | c                        |
| <i>Eno12</i>    | c                  | c                  | c <sup>87</sup> ,a | c                                   | c                                   | c                                   | c <sup>83</sup> ,b                                   | c                                   | c                                   | c                        |
| <i>Est1</i> *   | b                  | b                  | b                  | b                                   | b                                   | b                                   | b  | b                                   | b                                   | a                        |
| <i>Est2</i> *   | a                  | a                  | a                  | a                                   | a                                   | a                                   | a  | a                                   | a                                   | b                        |
| <i>Fdp</i>      | b <sup>67</sup> ,a | a <sup>50</sup> ,b | b <sup>62</sup> ,a | b <sup>62</sup> ,a                  | b                                   | b                                   | b  | b                                   | b                                   | a                        |
| <i>Fum</i> *    | b                  | b                  | b                  | b                                   | b                                   | b                                   | b  | b                                   | b                                   | a                        |
| <i>G6pd</i> *   | a                  | a                  | a                  | a                                   | a                                   | a                                   | a  | a                                   | a                                   | b                        |
| <i>Got1</i> *   | b                  | b                  | b                  | b                                   | b                                   | b                                   | b  | b                                   | b                                   | a                        |
| <i>Got2</i> *   | a                  | a                  | a                  | a                                   | a                                   | a                                   | a  | a                                   | a                                   | b                        |
| <i>Gpi1</i>     | b <sup>67</sup> ,a | a <sup>50</sup> ,b | b                  | b <sup>87</sup> ,a                  | b                                   | b                                   | b  | b                                   | b                                   | b <sup>94</sup> ,c       |
| <i>Gpi2</i> *   | b                  | b <sup>67</sup> ,c | b <sup>50</sup> ,c | b <sup>75</sup> ,a <sup>13</sup> ,c | e <sup>60</sup> ,c <sup>30</sup> ,b | e <sup>50</sup> ,c <sup>33</sup> ,a | c <sup>33</sup> ,d <sup>33</sup> ,a <sup>17</sup> ,e | e <sup>66</sup> ,c <sup>17</sup> ,a | c <sup>60</sup> ,a <sup>20</sup> ,e | f                        |
| <i>Lap</i> *    | b                  | b                  | c                  | c                                   | c                                   | c                                   | c  | c                                   | c                                   | a                        |
| <i>Ldh2</i>     | b                  | b                  | b                  | b                                   | b <sup>90</sup> ,a                  | b                                   | b  | b                                   | b                                   | b                        |
| <i>Mdh2</i>     | a                  | a                  | a                  | a                                   | a <sup>90</sup> ,b                  | a                                   | a <sup>67</sup> ,c                                   | a                                   | a <sup>90</sup> ,b                  | a                        |
| <i>Me1</i> *    | b <sup>83</sup> ,a | b                  | b                  | b                                   | b                                   | b                                   | b  | b                                   | b                                   | c                        |
| <i>Me2</i>      | b                  | b                  | b <sup>87</sup> ,a | b                                   | b                                   | b                                   | b  | b                                   | b                                   | b <sup>75</sup> ,c       |
| <i>Pep-A1</i>   | a                  | a                  | a                  | a                                   | a <sup>90</sup> ,b                  | a                                   | a  | a                                   | a                                   | a                        |
| <i>Pep-A2</i>   | b                  | b                  | b                  | b <sup>87</sup> ,a                  | b                                   | b                                   | b  | b                                   | b                                   | b                        |
| <i>Pep-B</i> *  | a                  | a                  | a                  | a                                   | a                                   | a                                   | a  | a                                   | a                                   | b                        |
| <i>Pep-D1</i> * | a                  | a                  | a                  | a                                   | a                                   | a                                   | a  | a                                   | a                                   | b                        |
| <i>Pep-D2</i>   | b                  | b                  | b                  | b                                   | b <sup>90</sup> ,a                  | b                                   | b  | b                                   | b <sup>90</sup> ,a                  | b                        |
| <i>6Pgd</i> *   | c                  | c                  | c <sup>87</sup> ,a | c                                   | c                                   | c                                   | c  | c                                   | c                                   | b                        |
| <i>Tpi2</i>     | c <sup>83</sup> ,b | c                  | c <sup>87</sup> ,a | c <sup>87</sup> ,a                  | c                                   | c                                   | c  | c                                   | c                                   | c                        |



**FIGURE 5.** Type locality of *Nannoperca pygmaea* at the confluence of the Mitchell and Hay Rivers.

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