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Cyanea capillata is not a cosmopolitan jellyfish: morphological and molecular evidence for *C. annaskala* and *C. rosea* (Scyphozoa : Semaeostomeae : Cyaneidae) in south-eastern Australia

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Abstract. The taxonomic status of the lion's mane jellyfish, *Cyanea*, of south-eastern Australia has been unsettled since 1884 when medusae from Port Jackson were described as a new variety of *C. annaskala* von Lendenfeld rather than assigned to *C. rosea* Quoy & Gaimard described previously from the same location. *Cyanea annaskala* was later combined with *C. mullerianthe* Haacke then synonymised with *C. capillata* (Linnaeus), which is now considered a circumglobal species, before being resurrected as a subspecies, *C. capillata annaskala*, in 1986. Here I demonstrate that *Cyanea* in southern New South Wales and *Cyanea* in Tasmania and Victoria constitute two distinct morphological groups separated by >10% sequence difference in both cytochrome *c* oxidase subunit I and internal transcribed spacer 1. Moreover, these clades are molecularly distinct (>6%) from *C. capillata* collected in its North Sea type locality. Analyses of medusae from another type locality, Port Philip Bay, Victoria, demonstrate that *Cyanea annaskala* von Lendenfeld is a valid species. *Cyanea rosea* is tentatively resurrected for medusae from New South Wales, pending confirmation by analyses of medusae from the vicinity of Sydney. Assigning other south-eastern Australian *Cyanea* specimens from museum collections to species is difficult in the absence of molecular analyses because biogeographic and morphological inferences sometimes conflict. Integrative molecular and morphological analyses of medusae from type localities may offer the most robust approach to straightening out the often convoluted systematics of scyphomedusae.

Introduction

Medusae in the genus *Cyanea* are conspicuous members of the gelatinous zooplankton in many parts of the world, particularly in neritic temperate and polar waters (von Lendenfeld 1884*a*; Mayer 1910; Kramp 1961). *Cyanea capillata* (Linnaeus), the type species, was described from the North Sea (Linnaeus 1746; Linnaeus 1758) and later considered to have a circum-Arctic (Mayer 1910), amphitropical (Stiasny and van der Maaden 1943), or even global (Kramp 1961) distribution. As such, many subsequently described species of *Cyanea*, including *C. rosea* Quoy & Gaimard, 1824 from Port Jackson (Sydney) and *C. annaskala* von Lendenfeld, 1882 from Port Phillip (Melbourne), were synonymised as *C. capillata* (Stiasny and van der Maaden 1943; Kramp 1961, 1965).

The taxonomic history of south-eastern Australian *Cyanea* is thus complex (Table 1), but may be divided into three main periods. The first, from *c.* 1750 to *c.* 1900, was a period of species discovery and description (e.g. Linnaeus 1746; Haacke 1887). The second, from *c.* 1900 to *c.* 1965, was a period of synonymisation of 'the numerous so-called species of *Cyanea* [that] intergrade to such a degree … we can not maintain them' (Mayer 1910: 596); the evidence came from studies that found putatively diagnostic characters (such as pit-like intrusions of the gastrovascular system into the folds of the coronal muscle in *C. capillata* (Stiasny and van der Maaden 1943)) in taxa previously thought to lack them (such as *C. annaskala*; see Larson 1986). In some cases, local varieties were still recognised; for example, a lion's mane jellyfish in Port Jackson that matched von Lendenfeld's (1884*a*) description of *C. annaskala marginata* was identified as *C. capillata* var. *annaskala* (Pope 1949). In other cases they were not: after examining material from Port Philip, Kramp (1965) felt he could 'safely state' that *C. annaskala* was a synonym of *C. capillata*.

The third period, one of disentanglement, began when Russell (1970) suggested *C. annaskala* was distinct from *C. capillata*. Subsequently, Larson (1986) recognised the subspecies, *C. capillata annaskala*, from southern Australia, New Zealand and the Auckland Islands. Most recently, nuclear *5.8S* and partial *28S* rDNA sequence data indicated species-level differences between boreal and antipodean *Cyanea* (see Dawson 2004).

In light of the long-term, continuing, uncertainty regarding the validity of *C. annaskala* and recent molecular and

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morphometric analyses that have clearly demonstrated multiple 'cryptic' species in widespread scyphozoans (Dawson and Jacobs 2001; Dawson 2004; Holland *et al*. 2004), reconsideration of the status of the lion's mane jellyfish, 'snottie' or 'hair jellyfish' (Southcott 1982), as it is commonly known in Australia, is warranted. Here, I re-examine the status of *Cyanea* in south-eastern Australia using new material collected in Tasmania and New South Wales and older specimens housed in south-eastern Australian museums. I report traditional morphologic, new morphometric, and molecular results to establish the foundations for a more stable taxonomy for *Cyanea* in the region.

Materials and methods

Collections and material examined

Five *Cyanea* medusae were collected in plastic bags at the waters' edge of Merimbula Lake (New South Wales, 23.xii.2002; see Australian Museum lots G16864 [1 specimen] and G16865 [3 specimens]) and six from a small boat on the Huon Estuary (Tasmania, 03.ii.2003; see Australian Museum lots G16860 [2 specimens], G16861 [2 specimens], G16862 [1 specimen], and G16863 [1 specimen]). These were the only two sites at which medusae were found during collecting trips that visited multiple sites along the south-east coast of mainland Australia between Sydney and Melbourne and also the Tamar Estuary in Tasmania (Fig. 1). *Cyanea* were not found in Port Jackson or Botany Bay despite over a dozen visits to one or both locations between June 2001 and December 2004. Oral arm tissue was clipped from collected specimens and preserved in 90% ethanol before preservation of each medusa in 4% formalin in seawater. A single 2–3 cm bell-diameter medusa from Port Phillip Bay (Melbourne, Workshops Pier, approx. 37°51′S 144°54′W; 16.iv.2004) was preserved in 70% ethanol. Tissue samples, preserved in DMSO+NaCl (Dawson *et al*. 1998) for DNA analyses, were also obtained from *C. capillata* in Raunefjorden (Norway, 60°13′N 5°16′E; 26.vi.2000, 28.vii.2000) and Kachemak Bay (off Jakolof Bay dock, Alaska; 3.vi.2000). Morphological analyses also considered specimens from the Australian Museum (G15738 [Cairns, Queensland, 1 medusa identified as a member of the *Cyanea 'nozakii'* group by R. Condon], G13413 [Botany Bay, New South Wales, 3 medusae], G15739 [D'Entrecasteaux Channel, Tasmania, 3 medusae], G13710 [Flinder's Island, Tasmania, 1 medusa]), and Museum Victoria (F81974 [Port Phillip, Victoria, 2 medusae], F81882 [Bittangabee, New South Wales, 1 medusa], F81482 [Port Phillip, Victoria, 1 medusa], F81504 [Port Phillip, Victoria, 1 medusa], F81506 [Port Phillip, Victoria, 1 medusa], F73903 [Port Phillip, Victoria, 1 medusa], F81480 [Port Phillip, Victoria, 2 medusa]).

Molecular analyses

DNA was extracted from ~10 mm^3 ethanol-preserved oral arm tissues using a CTAB-phenol/chlorofom-based protocol (Dawson *et al*. 1998; Dawson and Jacobs 2001) and resuspended in 70 μL 10 mM TRIS-HCl pH 8.3. Cytochrome *c* oxidase subunit I (*COI*) was amplified using primers LCOjf (5′-ggtcaacaaatcataaagatattggaac; Dawson 2005*a*) and HCO2198 (5′-taaacttcagggtgaccaaaaaatca; Folmer *et al*. 1994). Internal transcribed spacer 1 (*ITS1*) was amplified using primers jfITS1-5f (5′-ggtttccgtaggtgaacctgcggaaggatc) and jfITS1-3r (5′-cgcacgagcc-gagtgatccaccttagaag; Dawson and Jacobs 2001). In each 50 μL reaction, primers (Invitrogen, Carlsbad, CA, USA) were at final concentration 0.5 μM, with 0.5 U Taq polymerase, 5 μL 10× buffer, 3 mM $MgCl_2$ (Applied Biosystems, Foster City, CA, USA), 0.2 mM dNTPs (Bioline, Sydney, Australia), and 1 μL purified DNA. All PCRs consisted of six steps of 94°C for 8 min, 49°C for 2 min, 72°C for 2 min, 94°C for 4 min, 50°C for 2 min, 72°C for 2 min, then 33 cycles of 94°C for 45 s, 51°C for 45 s, and 72°C for 60 s, followed by an extension step at 72°C for 10 min; the reaction was terminated by cooling to 4°C. PCR products were purified (*COI* using Qiagen (Melbourne, Australia) PCR clean-up columns; *ITS1* using Invitrogen's TOPO TA cloning kit and Pharmacia's Flexiprep kit (Piscataway, NJ, USA)), labelled with BigDye and sequenced on ABI 377 automated sequencers according to the maker's protocols (Applied Biosystems). Electropherograms were checked visually, misreads corrected, and poorly resolved terminal portions of sequences discarded. The identities of sequences were confirmed by BLAST searching sequences in GenBank (Altschul *et al*. 1997) and, for *COI*, establishing the existence of an open reading frame by applying the *Drosophila* mitochondrial code translation in DNA Strider 1.2 (Marck, Service de Biochimie et de Génétique Moléculaire, Direction des Sciences de la Vie, CEA, France). Ribosomal DNA sequence data for *Cyanea* sp. (U65481; Odorico and Miller 1997) from eastern USA (P. Anderson, personal communication) was downloaded from GenBank. Homologous sequences from *Aurelia* (*COI*: AY903096,

Table 1. Summary of major changes in taxonomy, and deduced biogeography, of *Cyanea* species reported from south-eastern Australia

Source	*C. annaskala*	*C. capillata*	*C. rosea*
Linnaeus (1746, also see 1758)		sp. nov. (*Medusa capillata*, North Sea)	
Péron and Lesueur (1809)		*Cyanea* gen. nov.; *C. capillata* as type species	
Quoy and Gaimard (1824)			sp. nov. (Port Jackson)
von Lendenfeld (1882)	sp. nov. (Port Philip)		
von Lendenfeld (1884*a*, 1884*b*)	var. *purpurea* (Port Philip) var. *marginata* (Port Jackson)		Moved to genus *Stenoptycha*
Mayer (1910)	Senior synonym of *C. mullerianthe* Haacke; temperate Australia	North Atlantic, North Pacific	Synonym of *C. annaskala*
Stiasny and van der Maaden (1943)	Synonym of *C. capillata*	North Atlantic, Australia	Doubtful species
Pope (1949)	Subspecies *C. capillata annaskala*		
Kramp (1961, 1965)	Synonym of *C. capillata*	Almost cosmopolitan in arctic and temperate seas	Doubtful species
Russell (1970)	Status uncertain	North Atlantic, Pacific	
Larson (1986)	Subspecies of *C. capillata*; southern Australia, New Zealand		
This study	Valid species, Maugean (Fig. 1)	North Atlantic, North Pacific	Probably valid, Peronian (Fig. 1)

AY903133; rDNA: AY319836, AY935212) and *Chrysaora* (*COI*: DQ083524; *ITS1*: DQ083525) were included as outgroups because, as the representatives of the two other families (Ulmaridae and Pelagiidae respectively) in Semaeostomeae for which *COI* and *ITS1* have been sequenced, they are phylogenetically the most appropriate. *COI* sequences were aligned on the basis of the amino acid translations; *ITS1* sequences were aligned in ClustalX (Jeanmougin *et al*. 1998) and then corrected by eye. All positions with missing data were excluded from subsequent analyses.

The *COI* and *ITS1* gene trees were constructed using maximum parsimony (MP) and maximum likelihood (ML) optimality criteria in PAUP* 4.0b10 (Swofford 2002). The MP analyses employed the branch-and-bound search algorithm on 'unweighted' and, for *COI*, weighted (changes weighted as the inverse of the relative frequencies of transitions and transversions, and of variable 1st, 2nd, and 3rd positions) datasets; gaps were coded as a 5th state. The ML analyses were heuristic searches using random sequence addition (1000 replicates, saving one tree per replicate) and tree bisection–reconnection (TBR) branch swapping; substitution models were fitted to the data using ModelTest (Posada and Crandall 1998). The phylogeny of *Cyanea* was subsequently estimated using combined *COI* and *ITS1* data. This 'total evidence' (Kluge 2004) analysis employed: (*1*) unweighted MP of the concatenated *COI* and *ITS1* datasets including specimens for which only one marker was available; and (*2*) ML analyses of only those specimens for which both markers were available. Bootstrap analyses of all datasets were run for 10 000 (MP) or 1000 (ML) realisations.

Morphological analyses

Sixteen morphological features used previously in the systematic literature on *Cyanea* were potentially useful for comparative analyses and were studied herein (Fig. 2). Two features were excluded after preliminary measurements owing to difficulties accurately enumerating the number of tentacle rows (*ntr*) and the number of tentacles (*nt*). Data on the remaining 14 features were gathered from the Huon Estuary, Merimbula Lake, and museum specimens described above.

Throughout, I refer to 'features' in their context as the raw observations from which 'characters' might subsequently be selected (Fristrup 1992: 45). Each independent feature and correlated set of features (or 'feature complex' to paraphrase Ghiselin 1997) observed in such a sample of organisms potentially is a 'character' (Fristrup 1992: 45). The final choice of characters, however, may be influenced by analytical methods and the perceptions of the investigator (Fristrup 1992: 50), as evident in literature on *Cyanea* and other scyphomedusae (e.g. *Aurelia*: Mayer 1910; Kramp 1968; Greenberg *et al*. 1996; Dawson and Jacobs 2001; Gershwin 2001) and, as such, the criteria for choosing characters should be as explicit and objective, and preferably quantitative, as possible (Wiens 2001). Because the sampling of *Cyanea* populations and species for this study was unavoidably limited, precluding detailed analysis of character status (e.g. Dawson 2005*b*), I refrain from assigning the status of 'character' to any 'feature', or complex thereof, discussed herein.

Correlation of quantitative (or 'continuous') features (i.e. mass (in g); *bt*, *ncn*, *ncmf*, *dcmf*, *nrmf*, *dpmc*, *dsmc*, *ntr*, *nt*, *nsl* (dimensions in mm); Fig. 2) with bell diameter was tested using Pearson's correlation. The *t*-test was used to compare Merimbula Lake and Huon Estuary medusae (the only samples with known genetic affinities and for which preservation was known to be similar) with regards to all quantitative features because bell diameter did not differ significantly between the two samples (t = -0.051, d.f. = 8, P = 0.960). Differences in categorical (or 'discrete') features (i.e. *oat*, *gvpc*, *gvpr*, *ncp*, *tmc*; Fig. 2) between Merimbula Lake and Huon Estuary samples were analysed using the χ^2-test.

Morphological similarity between all medusae was assessed by principal components analysis (PCA) of a subset of eight features (*gvpc*, *ncp*, *ncn*, *ncmf*, *dcmf*, *nrmf*, *dpmc*, *dsmc*, see Fig. 2) and 24 medusae for which most data were available. Values for all features

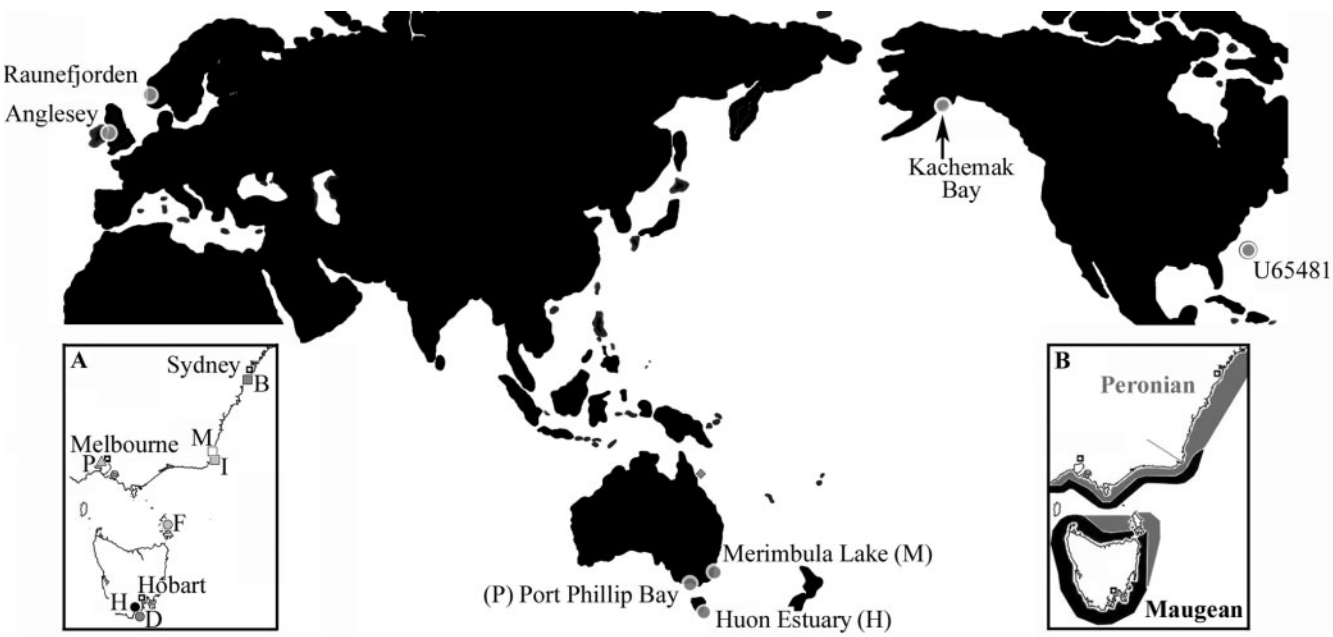

Fig. 1. (Main), locations at which *Cyanea* was collected for genetic analyses; single-letter codes refer to inset A. (Inset A), detail of south-eastern Australia showing collection localities of museum specimens used in morphological analyses: B, Botany Bay; D, D'Entrecasteaux Channel; F, Flinders Island; I, Bittangabee; P, Port Phillip. The Huon Estuary (H) and Merimbula Lake (M, actually an estuary and harbour as opposed to a lake), which were also included in morphological analyses, are shown for reference. Cairns is indicated by a grey diamond on the main map; (Inset B), the biogeography of south-eastern Australia following Bennett and Pope (1953).

were first expressed as a ratio of bell diameter (except *gvpc*, *ncp*, *ncmf*, *dcmf*, which were not correlated with bell diameter), then rescaled between 0 and 1 by dividing each observed value by the maximum value observed for that feature. All values were increased by one to differentiate values <1 from those that were missing, and features classed as nominal, ordinal, or numerical. Missing values were excluded from analyses. The object principal was optimised to maximise resolution of distances between medusae. Owing to uncertainties regarding the presence or absence of gastrovascular pits in the coronal muscle, PCA was repeated including *gvcp*, excluding *gvcp*, and coding *gvcp* as missing in all museum specimens. All analyses were completed using SPSS version 10 for Macintosh (SPSS Inc., Chicago, IL, USA).

Results

Molecular analyses

Phylogenetic analyses of *COI* and *ITS1*, independently and combined, revealed three reciprocally monophyletic clades of *Cyanea*: (*1*) *C. capillata* from Anglesey (UK), Norway, and Alaska; (*2*) putative *C. annaskala* from Tasmania and Victoria, including the type locality Port Phillip; and (*3*) medusae from New South Wales preliminarily identified as *C. rosea* (Fig. 3). *COI* sequences obtained from tissue samples of Alaska *Cyanea* were indicated by more extensive phylogenetic analyses of *COI* data (not shown) to be from an hydrozoan associated with the Kachemak Bay *Cyanea* rather than from the *Cyanea* itself and so are not reported here. All analyses involving *ITS1* also distinguished GenBank sequence U65481 representing eastern North America *Cyanea* from all of the aforementioned clades (Fig. 3). Mean percentage sequence divergence between clades of *Cyanea* varied from 11.8% to 15.3% in *COI* and from 5.8% to 11.2% in *ITS1* (Table 2). Although clades of *Cyanea* were well resolved, their interrelationships were not. Unweighted MP analyses of *ITS1* and ML analyses of *COI* paired *C. capillata*

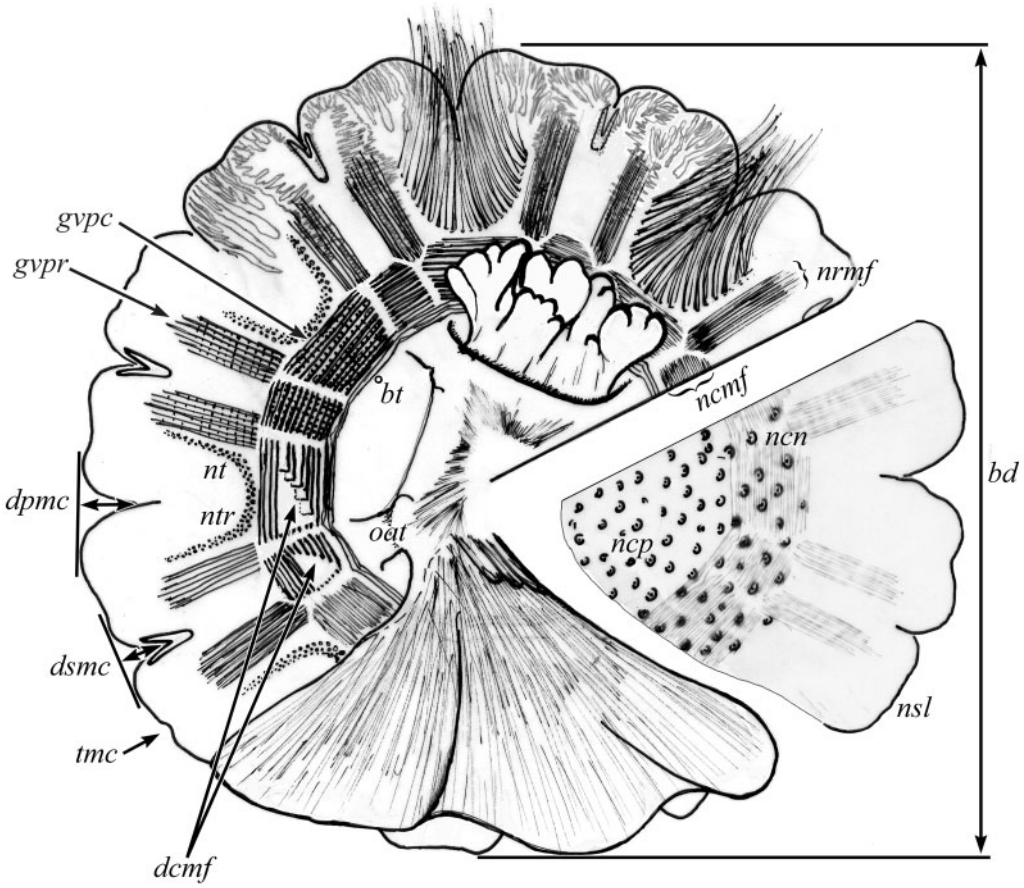

Fig. 2. (Main), schematic subumbrella perspective of *Cyanea* medusa with oral arms, gonads and tentacles variously cut away to show details of the morphological features considered. (Inset), schematic exumbrella perspective: bell diameter (*bd*); mean bell thickness at proximal edge of coral muscle band (*bt*); oral arms thickened at base (oat; boolean); gastrovascular pits in coronal muscle folds (*gvpc*; boolean); gastrovascular pits in radial muscle folds (*gvpr*; boolean); nematocyst clusters on exumbrella protrude (*ncp*; boolean); number of nematocyst clusters on exumbrella (*ncn*); mean number of coronal muscle folds per group (i.e. excluding circumbrellar coronal muscles; *ncmf*); depth of coronal muscle folds (*dcmf*); mean number of radial muscle folds per group (*nrmf*); mean depth of primary marginal clefts (*dpmc*); mean depth of secondary marginal clefts (*dsmc*); tertiary marginal clefts (*tmc*; present on ≥1 lobe); number of tentacle rows (*ntr*); number of tentacles (*nt*); number of secondary lobes (*nsl*). All dimensions in millimetres. Measurements quantitative unless stated otherwise.

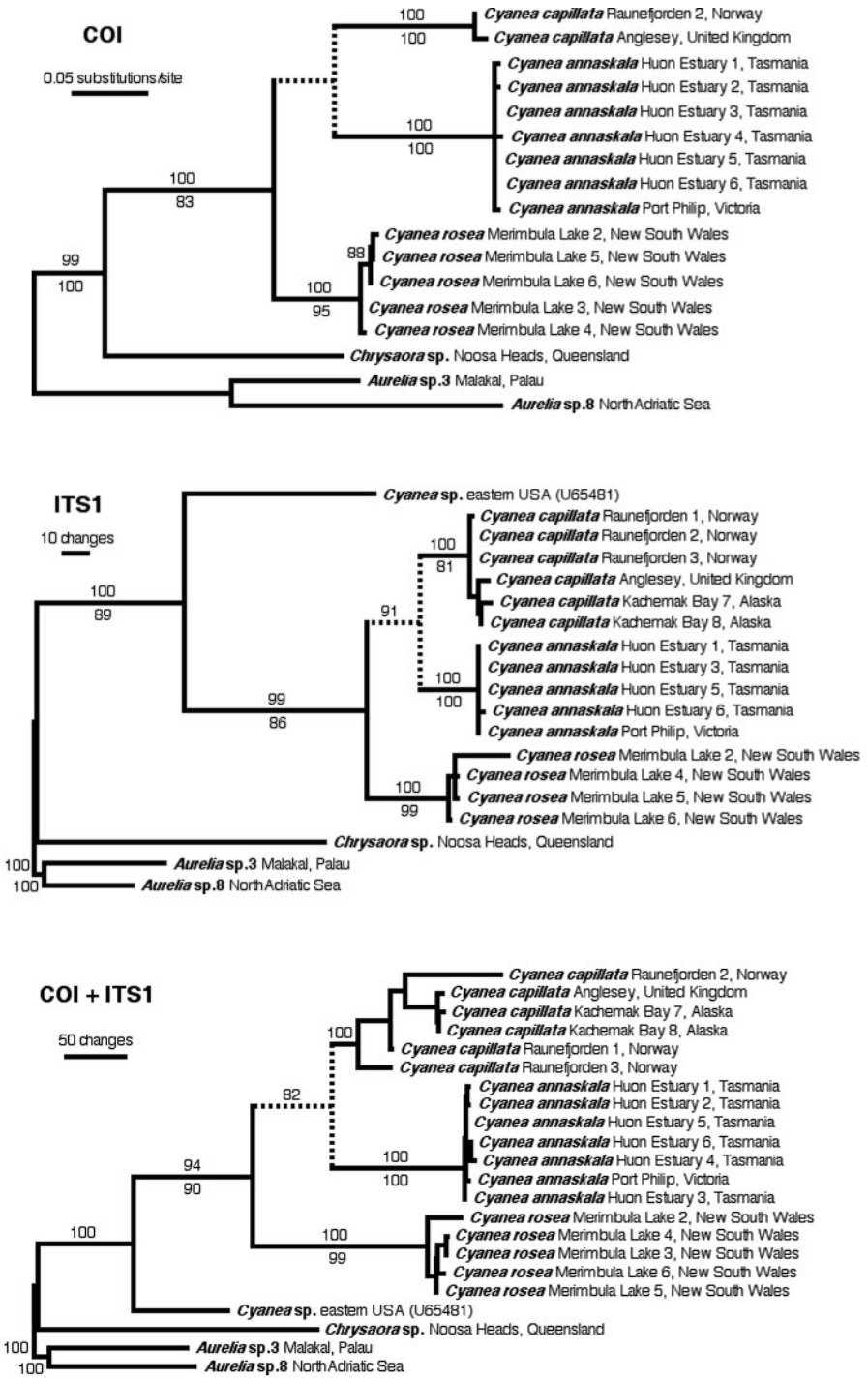

Fig. 3. (*a*) Single optimal cytochrome *c* oxidase subunit I (*COI*) gene tree constructed using maximum likelihood analysis (ML; –Ln L = 1836; heuristic search, random sequence addition (1000 replicates, save one tree per replicate) and TBR branch swapping) employing the GTR+I model. (*b*) Single optimal internal transcribed spacer 1 (*ITS1*) gene tree constructed using unweighted maximum parsiomony (UMP; branch-and-bound search) and gaps as a 5th character state (length = 452 steps, *CI* = 0.863). (*c*) *Cyanea* phylogeny based on combined analyses of *COI* and *ITS1*. Tree topology is from UMP analyses using the branch-and-bound search and including gaps as a 5th character state (length = 997 steps, *CI* = 0.807). Bootstrap values are shown above (UMP) and below (ML) branches; dashed branches show conflict between ML and MP analyses.

Table 2. Mean percentage pairwise sequence difference (± 95% *CI*) between clades of *Cyanea* in *COI* (upper triangle) and *ITS1* (gapped positions excluded; lower triangle)

Mean within-clade sequence differences are shown along the diagonal (*ITS1**COI*). Medusae are named (and location give parenthetically) if they were identified morphologically at the time of collection or if they were collected from a type locality; medusae from a known locality are named parenthetically if their identification was based on molecular and morphological analyses in this study

	Eastern USA	Kachemak Bay	*C. capillata*	Anglesey	Merimbula Lake	Huon Estuary	*C. annaskala*
Eastern USA (U65481)	– \ nd	nd	nd	nd	nd	nd	nd
Kachemak Bay (*C. capillata*)	6.09 ± 0.25	0.45 \ nd	nd	nd	nd	nd	nd
C. capillata (Norway)	5.82 ± 0.01	0.90 ± 0.14	– \ –	0.83 ± –	13.99 ± –	14.51 ± –	14.69 ± –
Anglesey (*C. capillata*)	6.51 ± –	1.52 ± 0.23	1.74 ± 0.01	– \ –	13.77 ± 0.47	11.84 ± 0.01	11.87 ± –
Merimbula Lake (*C. rosea*)	10.11 ± 0.37	6.46 ± 0.24	6.22 ± 0.27	7.71 ± 0.42	0.50 \ 0.70	15.20 ± 0.10	15.31 ± 0.25
Huon Estuary (*C. annaskala*)	5.92 ± 0.37	9.07 ± 0.20	9.10 ± 0.22	10.36 ± 0.52	10.84 ± 0.22	0.33 \ 0.29	0.37 ± 0.14
C. annaskala (Port Phillip)	6.01 ± –	9.20 ± 0.24	9.34 ± 0.01	10.09 ± –	11.22 ± 0.36	0.26 ± 0.51	– \ –

nd, No *COI* data; –, too few sequences to calculate.

with *C. annaskala* (Fig. 3*b*), whereas unweighted MP analyses of *COI* and ML analyses of *ITS1* paired *C. capillata* with *C. rosea*. MP and ML analyses of combined *COI+ITS1* datasets did not show strong support for either topology (Fig. 3*c*).

Morphological analyses

All tetramerous medusae possessed four perradial curtain-like lips, four-folded sack-like gonads, eight horseshoe-shaped clusters with several rows of tentacles arising from the floor of the subumbrella, no ring canal, and radial and circular muscle bands (or a subset thereof determinable in older museum specimens). The margin of all tetramerous medusae was divided by eight primary and eight secondary clefts, giving 16 velar lobes. On medusae in good condition it was possible to see that at least occasionally the lobes were further slightly indented giving two tertiary velar lappets. All medusae were identified as members of the genus *Cyanea*.

Measurement of museum specimens was more difficult than measurement of recently preserved specimens. Deterioration of tissue in museum specimens made it impossible to determine beyond doubt that gastrovascular pits were absent. It was difficult to enumerate the number of exumbrella warts on museum specimens because the warts were never large (cf. the bigger, more recently preserved, Merimbula Lake medusae) and the bell was often mishapen. However, the presence of exumbrellar nematocyst warts on three small (e.g. 75–90 mm bell diameter) museum specimens indicated, at least, that failure to find warts on other museum specimens was likely to be a true indication of their absence. Non-zero counts of nematocyst warts on these three small medusae were estimated from counts on portions of the bell accordingly scaled up. The mesoglea at the base of the oral arms of at least some medusae from Port Phillip (Museum Victoria specimens) was noticeably thickened, as in Merimbula Lake medusae (Table 3). The depth of coronal muscle folds of the *Cyanea* '*nozakii*'-group specimen was

Table 3. Pairwise comparisons of morphological features between cryptic species of *Cyanea* in the Huon Estuary and Merimbula Lake

All dimensions in millimetres; mass in grams; sample size (n) = 5 for both Huon Estuary and Merimbula Lake except mass (n_{HE} = 3, n_{ML} = 5), *ncn*, *dpmc* and *dsmc* (n_{HE} = 5, n_{ML} = 4)

	Huon Estuary (*C. annaskala*)		Merimbula Lake (*C. rosea*)				
	Mean	s.e.	Mean	s.e.	t	d.f.	P
Bell thicknes (*bt*)	20.6	0.7	21.8	0.8	–1.1	8	0.300
Mass	385.0	106.7	420	50.3	–0.3	6	0.745
Number of nematocyst clusters (*ncn*)	0.0	0.0	386	37.3	–10.3	3	0.002*
Number of coronal muscle folds (*ncmf*)	18.2	0.5	12.5	0.3	9.6	8	0.000*
Number of radial muscle folds (*nrmf*)	12.2	0.9	9.9	0.3	2.3	8	0.047
Depth of primary marginal clefts (*dpmc*)	18.1	1.2	18.3	0.6	–0.1	7	0.887
Depth of secondary marginal clefts (*dsmc*)	13.3	0.9	10.4	0.9	2.2	7	0.065
					χ^2		
Colour of oral arms	Purple throughout		Colourless				
Mesoglea at base of oral arms	Not thickened		Noticeably thickened				
Pits in coronal muscle folds (*gvpc*)	No		Yes		5.0	1	0.025†
Nematocyst clusters protrude (*ncp*)	No		Yes		5.0	1	0.025†
Depth of coronal muscle folds (*dcmf*)	Shallow (~1 mm)		Deep (~4 mm)		5.0	1	0.025†

s.e., Standard error; d.f., degrees of freedom. *Significant at P = 0.05 after sequential Bonferroni correction for seven tests. †not significant at P = 0.05 after sequential Bonferroni correction for three tests.

~10 mm, notably thicker than other measured specimens (Table 3).

Three features were correlated with bell diameter: mass (r = 0.960, P <0.001, n = 8; note that mass is expected to increase exponentially with bell diameter if a larger size range of *Cyanea* is studied), mean depth of primary marginal clefts (*dpmc*: r = 0.937, P < 0.001, n = 21), and mean depth of secondary marginal clefts (*dsmc*: r = 0. 0.862, P <0.001, n = 21). Another three features were correlated with bell diameter after minor manipulation of the datasets: bell thickness after excluding one extreme value (*bt*: r = 0.893, P = 0.001, n = 9), number of nematocyst clusters on exumbrella after excluding all zero values (*ncn*: r = 0.973, P <0.001, n = 7), and mean number of radial muscle folds after excluding the data point for the *C.* '*nozakii*'-group specimen (*nrmf*: r = 0.520, P = 0.019, n = 20). Only one quantitative feature, the number of coronal muscle folds (*ncmf*), was not correlated with bell diameter (Fig. 4). A subset of these features differed statistically between the genetically distinct Huon Estuary and Merimbula Lake medusae (Table 3), which were clearly also distinct considering all morphological features together (Fig. 5).

Discussion

The *Cyanea* collected in south-eastern Australia are genetically distinct from *C. capillata* collected in the North Sea

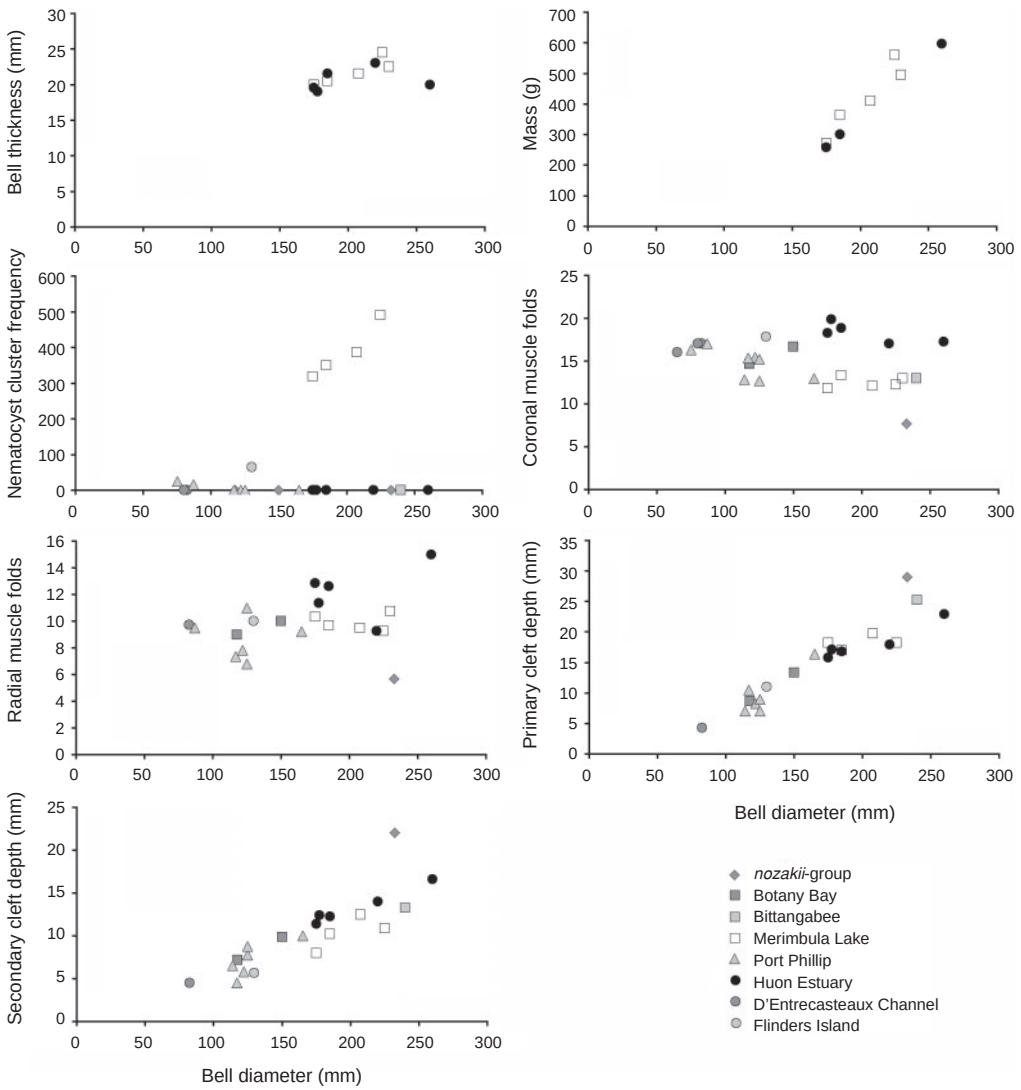

Fig. 4. Scatterplots showing morphological variation in *Cyanea* medusae from south-eastern Australia. Two populations have been distinguished on the basis of molecular analyses: Huon Estuary and Merimbula Lake. Museum specimens represent other populations in the region of unknown molecular affinity: Botany Bay, Bittangabee, D'Entrecasteaux Channel, Flinder's Island and Port Phillip. A medusa in the *Cyanea* '*nozakii*'-group from Queensland is included for comparison. All values plotted are the arithmetic mean of at least two measurements in different quadrants per medusa except those for mass (one measurement only).

type locality and Kachemak Bay, Alaska, and from *Cyanea* sampled from the eastern United States (Fig. 3). The degree of genetic difference is consistent with species-level differences in other scyphozoans (Table 2; Dawson and Jacobs 2001; Holland *et al*. 2004). *Cyanea* from south-eastern Australia are morphologically distinct from a Queensland *C*. '*nozakii*'-group medusa, representative of a group of western Pacific species (*C. buitendijki* Stiasny, *C. mjoebergi* Stiasny, and *C. nozakii* Kishinouye; see Kramp 1965), which has been reliably and consistently separated from *C. capillata*-like medusae since the morphological analyses of Stiasny and van der Maaden (1943; Kramp 1961, 1965). These results are consistent with prior studies that recognised taxonomically distinct medusae, generally identified as *Cyanea annaskala*, endemic to south-eastern Australia (e.g. von Lendenfeld 1882, 1884*a*; Mayer 1910; see also Larson 1986).

The new molecular and morphological analyses presented here further demonstrate that *Cyanea annaskala* is a 'complex' of two species in south-eastern Australia. One of these can be identified as *C. annaskala* sensu stricto because one specimen was sampled from the type locality, Port Phillip (von Lendenfeld 1882). On geographic (southern Victorian and Tasmanian waters) and morphological grounds (the oral arms of Huon Estuary medusae were heavily pigmented purple throughout) *C. annaskala* sensu stricto supercedes von Lendenfeld's (1884*a*) variety, *C. annaskala purpurea*. By reciprocity, medusae from Merimbula Lake are implicated as being synonymous with von Lendenfeld's (1884*a*, 1887) second variety: *C. annaskala marginata* from Port Jackson; Merimbula Lake medusae had concomitantly colourless oral arms and came from the same warm-temperate biogeographic province. The Merimbula Lake medusae, however, are more appropriately identified as *C. rosea* because these features, the medusae's 'verrucos[e]' exumbrella and, particularly two specimens from Merimbula Lake whose 'couleur générale [was] d'un beau rose', also fit Quoy and Gaimard's (1824) description, which has date priority over *C. annaskala* (see von Lendenfeld 1882, 1884*a*, 1887) with regard to Port Jackson. Medusae from other south-eastern Australian locations could tentatively be assigned to species on the basis of their morphologies (Fig. 4) or geographic and biogeographic affinities (Fig. 1) but conflicting inferences, most notably regarding Botany Bay medusae (Fig. 1 cf. Figure 5), suggest this would be premature without matching genetic data.

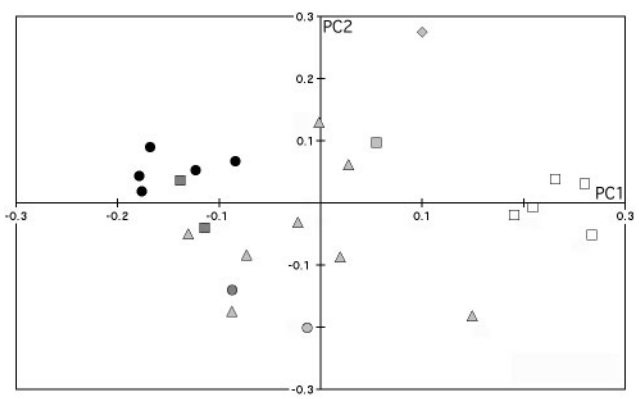

Fig. 5. Two-dimensional principal components analysis (PCA) plot indicating morphological similarity among *Cyanea* medusae from south-eastern Australia. PC1 explains 35% of observed variation (Cronbach's $\alpha = 0.80$). PC2 explains 18% of observed variation (Cronbach's $\alpha = 0.47$). Symbols as in Fig. 4. The same pattern was evident whether *gvcp* was excluded from, included in, or coded as missing in the analysis.

Reconciling modern statistical molecular phylogenetic and multivariate morphological analyses with traditional taxonomic descriptions has benefits beyond the immediate goal of correctly identifying species and their boundaries. One proximate reward is insight into the chain of events that can lead to classifications that misrepresent natural groups and divisions. In the case of south-eastern Australian *Cyanea*, problems began accruing in 1884, when von Lendenfeld, having moved *C. rosea* to the genus *Stenoptycha*, included medusae from Port Jackson as a variety of *C. annaskala* based, de rigueur, on a handful of qualitative macro-morphologic features such as a few protruding nettle-warts in the centre of the exumbrella (von Lendenfeld 1884*a*, 1884*b*). Notably, neither Quoy and Gaimard (1824) nor von Lendenfeld (1882, 1884*a*, 1884*b*, 1887) mentioned gastrovascular intrusions in the musculature, nor did Benham (1909) nor Mayer (1910). Yet the presence of gastrovascular pits was suggested as an important characteristic of *capillata*-group medusae by Stiasny and van der Maaden (1943) when they synonymised *C. annaskala* (= *C. annaskala* + *C. rosea*) with *C. capillata*. Kramp (1961, 1965) ignored the characteristic. Russell (1970), in contrast, thought it was important, listing the presence of gastrovascular pits as diagnostic of *C. capillata*. Larson (1986) disagreed: the absence of gastrovascular pits and presence of conspicuous nematocyst warts was diagnostic of the subspecies *C. capillata annaskala*, which ranged, he wrote, from southern Australia to New Zealand and the Auckland Islands. He came to this conclusion—one which clearly differs from the original description of *C. annaskala*—without examining any specimens (Larson 1986).

The human contribution to the instability in the taxonomy of *Cyanea* is unmistakable, but it is only one of many internecine factors. Original species descriptions are often vague but typological. Intra-specific variation, however, is pervasive (e.g. Brewer 1991). Yet, the natural temporal and spatial patchiness of medusae hinders thorough geographic sampling, so morphological variation within species is often overlooked, which can make it difficult to identify differences between species (Dawson 2003). Museum collections mitigate this problem, but many museum specimens,

Table 4. Polychotomous key to species of *Cyanea* in south-eastern Australia, compared with *C. capillata*

1. Bell diameter (*bd*)	~25 cm or larger	Usually 30–50 cm; maximum 100–200 cm	~25 cm or larger
2. Exumbrella papillae (*ncp*, *ncn*)	Absent	Centre smooth, periphery faintly papillose	Present
3. Colour	Arms purple throughout, bell deep reddish brown	Yellow ochre to reddish brown, rarely colourless	Arms colourless, bell faint reddish brown or colourless, gut/gonad pink or yellow
4. Base of oral arms (*oat*)	Usually not thickened	Thickened	Thickened
5. Number of coronal muscle folds (*ncmf*)	17–24	13–15	11–14
6. Depth of coronal muscle folds (*dcmf*)	Shallow (~1 mm)	Shallow (~0.5–2 mm)	Deep (~4 mm)
7. Pits in muscle folds (*gvpc*, *gvpr*)	absent	Present	Present
8. Distribution	Cold temperate south-eastern Australia	Northern boreal	Warm temperate south-eastern Australia
9. *COI* (similar to …)	AY902912–17, AY902923	AY902911, AY902924	AY902918–22
10. *ITS1* (similar to …)	AY903061–65	AY903053–56, AY903066	AY903057–60
	C. annaskala	***C. capillata***	***C. rosea***

especially the oldest and most valuable (i.e. the holotypes), are damaged. This necessitates new collections, sometimes from new localities, which because of the incumbent lack of detail must be related back to incomplete descriptions of medusae from sometimes different localities. New techniques are often applied to the new specimens, exacerbating the gulf between the original descriptions and the data now available. Seemingly the only constant is the type locality, although this is also often vague and alone will be confounded by sympatric species. Plus, even environments change, for example, from invasion by non-indigenous congeners (e.g. Dawson 2003). Yet invasive species should be recognisable using a combination of historical records, morphometric and genetic techniques, and careful analyses of natural patterns of advection. Type localities may thus be the best starting point of reference for a thoroughly updated suite of analyses.

From this perspective, the conclusions made here for *C. capillata* and *C. annaskala*, which are based on integrative morphological and genetic analyses of specimens from type localities, should be considered robust. The identification of *C. rosea*, in contrast, is tentative, pending analyses of *Cyanea* from the Sydney region—'à environ vingt lieues du port Jackson' (Quoy and Gaimard 1824).

Key to species

A polychotomous key to the species *C. annaskala*, *C. capillata*, and *C. rosea* is provided in Table 4. It is based on the description of *C. capillata* by Russell (1970) and corresponding measurements of *C. annaskala* and *C. rosea* made during this study. It is accurate for the two populations studied here. Its broader applicability depends on the states of the morphological features discussed being representative of each species beyond the populations studied, which as noted above may not always be the case. Sequence data may be the most robust character type at this stage and are therefore included in the key.

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