CSIRO PUBLISHING
www.publish.csiro.au/journals/mr *Molluscan Research*, 2004, **24**, 149–159

Discovery of the Indo-Pacific oyster *Hyotissa hyotis* (Linnaeus, 1758) in the Florida Keys (Bivalvia: Gryphaeidae)

Rüdiger Bieler[A,D], *Paula M. Mikkelsen*[B], *Taehwan Lee*[C] *and Diarmaid Ó Foighil*[C]

[A]Department of Zoology, Field Museum of Natural History, 1400 S Lake Shore Drive, Chicago, IL 60605, USA.
[B]Department of Invertebrates, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024-5192, USA.
[C]Museum of Zoology, University of Michigan, 1109 Geddes Avenue, Ann Arbor, MI 48109-1079, USA.
[D]To whom correspondence should be addressed. Email: bieler@fieldmuseum.org

Abstract

Recent collections from a shipwreck in ~30 m depth off the Florida Keys recovered an exceptionally large gryphaeid oyster that was identified on morphological grounds as *Hyotissa hyotis* (Linnaeus, 1758), a common constituent of Indian and Pacific Ocean near-shore faunas. This identification was confirmed by molecular characterisation: the Florida specimen had an almost identical large mitochondrial ribosomal subunit (16S) genotype to that obtained from a western Pacific (Guam) conspecific, differing in only two nucleotide positions that were heteroplasmic in the Guam specimen. Although this species has been variously cited to occur in the western Atlantic, careful examination of these records revealed them to be misidentifications of *Hyotissa mcgintyi* (Harry, 1985), originally described from south-eastern Florida. Because *H. hyotis* is much larger than any other regional oyster, it is unlikely to have been overlooked in earlier biotic surveys. It is therefore likely that this specimen, and another recently discovered off West Palm Beach, Florida, stem from a recent undocumented introduction to the western Atlantic.

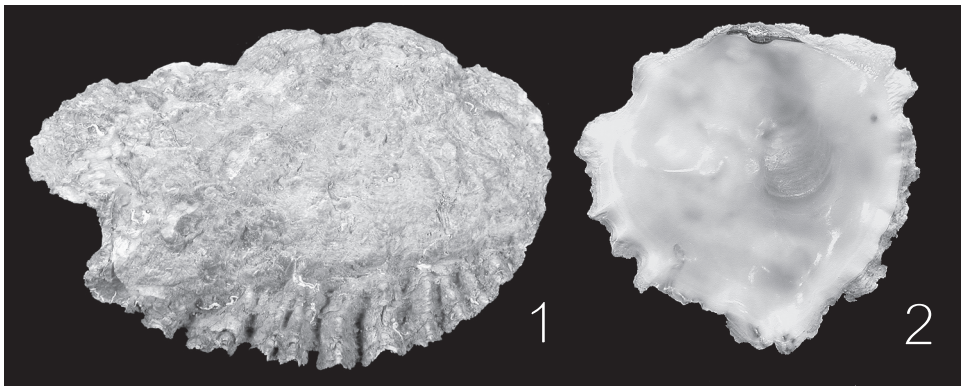
Additional keywords: 16S rDNA, heteroplasmy, introduced species, Mollusca, North America, western Atlantic.

Introduction

The western Atlantic is home to numerous oyster species, with members of the ostreid genera *Ostrea* Linnaeus, 1758 (introduced), *Crassostrea* Sacco, 1897, *Cryptostrea* Harry, 1985, *Dendostrea* Swainson, 1835, *Ostreola* Monterosato, 1884 and *Teskeyostrea* Harry, 1985, occurring off the western Atlantic coast of the United States (Turgeon *et al*. 1998; Kirkendale *et al*. 2004). Two species of Gryphaeidae have also been reported: *Neopycnodonte cochlear* (Poli, 1795) and *Parahyotissa mcgintyi* Harry, 1985 (Turgeon *et al*. 1998: 32; now placed in *Hyotissa* Stenzel, 1971, see below). Extant gryphaeids differ from ostreids in having more complex larval hinge dentition, a round adductor muscle scar, a ventricle that is penetrated by the rectum, a vesicular shell structure (that appears cellular or spongy under magnification), and so-called vermicular chomata, small rounded, sinuous shell ridges near the ligament on the anterior and posterior margins of both valves (Ranson 1941, 1967*a*, 1967*b*; Stenzel 1971; Torigoe 1981; Harry 1985).

Of the two recognised gryphaeids in this region, *Neopycnodonte cochlear* extends into deep water (with reports to 2100 m) and forms a white to pink to orange, thin, moderately sized (to 7 cm) shell comprised of a deeply cupped left and a flat right valve, and with a geographic range including the eastern and western Atlantic and the Indo-West Pacific (Harry 1986*a*; Carriker and Gaffney 1996). *Hyotissa mcgintyi* (Figs 1, 2) has a cream-coloured to lavender robust shell, more or less circular in outline, usually not exceeding 7–9 cm in height; a saw-toothed shell margin is often developed; its range

 10.1071/MR04013 1323-5818/04/030149



Figs 1, **2.** *Hyotissa mcgintyi*, Florida Keys (FMNH 302057). *1*, Outside of free valve, 87 mm greatest shell length; *2*, inside of free valve, 56 mm greatest shell length; note light-coloured margin.

includes the tropical eastern and western Atlantic (Carriker and Gaffney 1996), extending into the northern Gulf of Mexico and to North Carolina. A recent study involving nuclear (28S) and mitochondrial (16S) genes (Kirkendale *et al*. 2004) suggested that there is no phylogenetic basis for recognising *Parahyotissa* Harry, 1985, and proposed the placement of all hyotissinine taxa in the genus *Hyotissa* Stenzel, 1971, a recommendation here followed.

Hyotissa mcgintyi was originally described as *Ostrea thomasi* McLean, 1941, from a single specimen dredged off Palm Beach, south-eastern Florida. It was subsequently considered a synonym of the Indo-Pacific *Hyotissa* (or *Pycnodonte*) *hyotis* (Linnaeus, 1758) by various authors, including Abbott (1974). The name of the supposed senior synonym, *Hyotissa hyotis*, thus became generally used for the larger gryphaeid in the western Atlantic (e.g. Odé 1980). Also known as the honeycomb oyster or giant coxcomb oyster, *Hyotissa hyotis* is one of the largest oysters in the Indo-Pacific, reaching a shell diameter of ~30 cm (Saville-Kent 1893). It is the type species of *Hyotissa*.

In a review of worldwide oyster classification, Harry (1985) introduced a new generic name, *Parahyotissa*, for *Ostrea thomasi.* Recognising that the latter name was preoccupied by *Ostrea sellaeformis* var. *thomasii* Glenn, 1904 (a *nomen nudum* by Conrad, from the early Miocene of Maryland), he introduced a new name, *Parahyotissa mcgintyi*, for the species, and demonstrated morphological/anatomical differences between it and the Indo-Pacific *H. hyotis*. Harry (1985, 1986*a*, 1986*b*) re-identified western Atlantic records of larger-shelled gryphaeids (including those of Odé 1980) as belonging to *P. mcgintyi* and regarded *H. hyotis* as restricted to the Indo-Pacific. Subsequent lists thus excluded *H. hyotis* from the United States coastal fauna (Turgeon *et al*. 1998) and from the western Atlantic oyster fauna (Carriker and Gaffney 1996). The species was not found in a multi-year field/literature/collections survey of Florida Keys bivalves (Mikkelsen and Bieler 2000), and was not collected during a recent (2002) targeted oyster study in the middle Florida Keys by Kirkendale *et al*. (2004).

Nevertheless, the name *Hyotissa hyotis* had remained in post-1985 use in the western Atlantic fauna. Some sources, such as popular shell books and shell dealer listings, continued maintaining it as a cosmopolitan/circumtropical species. This might in part stem from Abbott's earlier (1974) synonymy of *Ostrea thomasi* (= *H. mcgintyi*) under the name of the Indo-Pacific form. In all cases in which we were able to confirm the identity of the

material in question, Atlantic records have been based on individuals of *H. mcgintyi*, not *H. hyotis*. A case in point is Rosenberg's (1992: 138) reference to *H. hyotis* as having circumtropical distribution: the Atlantic record is based on a specimen of *H. mcgintyi* (1992: 138, lower right figure; Grand Bahama Island, ANSP 371816; G. Rosenberg, personal communication). Published records of *H. hyotis* from the Brazilian coast, e.g. that of Celso Guimarães Prado (1996) from Maranhão State, and specimens acquired under that name from Brazilian shell dealers (FMNH 302056) from Bahia State, are likewise specimens of *H. mcgintyi*. The same is the case with the Cuban record of *H. hyotis* by Espinosa and Juarrero (1989), which was subsequently (Espinosa *et al*. 1994) corrected as referring to *H. mcgintyi*. Mexican records of *H. hyotis* refer to material from Baja California Sur (Sevilla-H. *et al*. 1998) and this species has long been documented as a member of the eastern Pacific fauna, previously as *Ostrea fischeri* Dall, 1914 (now regarded as a junior synonym of *H. hyotis* (see Harry 1985)).

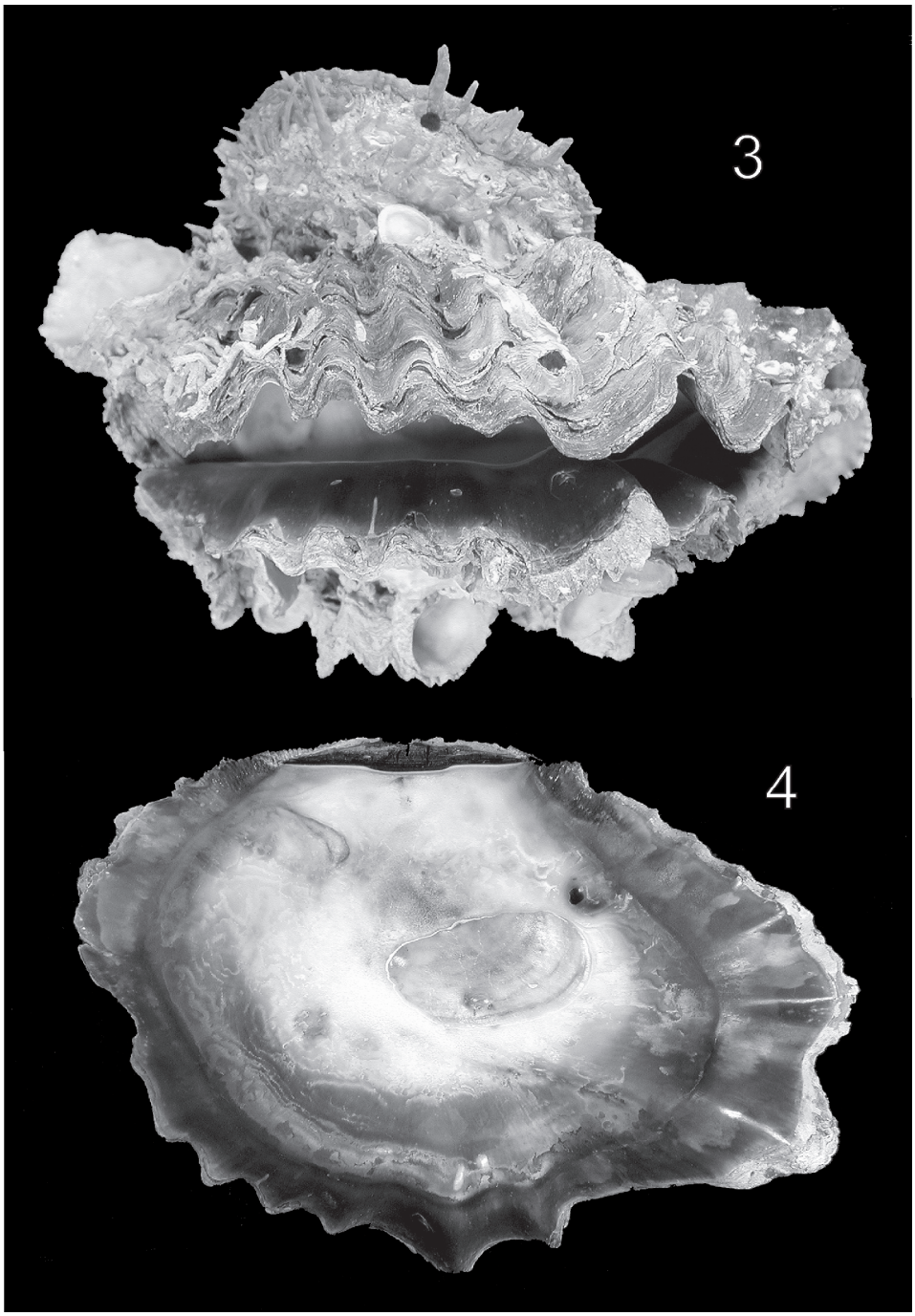
Recent collecting of cemented bivalves on a shipwreck off the middle Florida Keys brought to light an exceptionally large living oyster (Figs 3, 4; 18 cm greatest shell length) that differed greatly from the simultaneously collected *H. mcgintyi* specimens in its size, shell thickness, black shell pigmentation, and black mantle tissue. Its overall morphological appearance is very close to that of *H. hyotis* specimens from the Indo-Pacific and prompted revisiting the question of *H. hyotis*' presence in the western Atlantic. This note serves to establish its species-level identity by molecular phylogenetic analysis and to explore distinguishing shell characters between the two western Atlantic *Hyotissa* species (Table 1).

Materials and methods

The *Hyotissa hyotis* specimen (FMNH 302010) was collected during a scuba survey of bivalves that were part of the fouling community on the steel wreck of the research vessel 'Thunderbolt', off the middle Florida Keys (station FK-717, 19 Aug. 2003, about six nautical miles south of Marathon, 24°39.68′N, 80°57.82′ W, 29–35 m, R. Bieler, A. Bieler & P. Sierwald, coll.; same site collected as station FK-650 on 27 July 2002). The 200-foot ship was intentionally sunk on 6 March 1986, as part of the Florida Keys Artificial Reef Association project, and now lies intact and upright on a sand bottom in ~37 m of water. The specimen of *H. hyotis* formed the basis of a large cluster of fouling and encrusting organisms, including a large living specimen of *Spondylus americanus* Hermann, 1781, and numerous living individuals of *H. mcgintyi* and *Chama congregata* Conrad, 1833. Comparison was made with Indo-Pacific specimens of *H. hyotis* in the AMNH and FMNH collections.

A 95% ethanol-preserved tissue sub-sample of the Floridian *Hyotissa hyotis* specimen (FMNH 302010) was forwarded to the Museum of Zoology, University of Michigan. Total genomic DNA was obtained from 20–30 mg of mantle tissue using a Qiagen extraction kit (Valencia, CA, USA), from which a 492 nucleotide fragment of the mitochondrial large subunit ribosomal gene (16S) was amplified via the polymerase chain reaction using the Kessing *et al*. (1989) 'universal' primers 16Sar (5′-CGCCTGTTTATCAAAAACAT-3′) and 16Sbr (5′-GCCGGTCTGAACTCAGATCACGT-3′). A touchdown protocol (Palumbi 1996) was used: after 4 min denaturation at 94°C, the initial annealing temperature of 65°C was decreased by 2°C/cycle (40 s denaturing at 94°C, 40 s annealing and 1.5 min extension at 72°C) until the final annealing temperature (50°C) was reached and subsequently maintained for an additional 30 cycles. The PCR product was gel-purified (1% agarose) and a sequencing template was prepared using a QIAEX QXII Gel Extraction Kit (Qiagen). Direct, cycle sequencing reactions were performed using BigDye Terminator Cycle Sequencing Ready Reaction (Perkin-Elmer/Applied Biosystems, Palo Alto, CA, USA) with the respective original PCR primers for both strands of the amplified product. Sequencing products were electrophoresed at the University of Michigan Sequencing Core Facility. Resulting chromatograms were edited manually by comparing both strands for all taxa using Sequence Navigator 1.0.1 (Applied Biosystems).

The Floridian *Hyotissa hyotis* 16S mitochondrial genotype was added to a pre-existing gryphaeid 16S nexus file (Kirkendale *et al*. 2004), which included a conspecific haplotype from the western Pacific (Fig. 5), after alignment using Clustal X (Thompson *et al*. 1997). This dataset is available upon request from D. Ó Foighil (diarmaid@umich.edu). It was phylogenetically analysed with PAUP* ver. 4.0b10 (Swofford 2002) using the maximum parsimony optimality criterion with *Neopycnodonte cochlear* as the designated



Figs 3, **4.** *Hyotissa hyotis*, Florida Keys, 180 mm specimen (FMNH 302010). *3*, Gaping, partly cleaned shell with attached bivalves (*Spondylus americanus* above, *H. mcgintyi* on left and right, *Chama congregata* below); specimen was cemented to ship hull by the hinge region of the upper valve in this image; *4*, inside of free valve; note dark margin and moiré lines on the slightly iridescent surface.

Table 1. Taxonomic framework, locality data, and voucher specimen information for gryphaeid taxa used in this study

Higher grouping	Species	Taxonomic relevance	Sample locality	Museum vouchers	GenBank number
Pycnodonteinae: Hyotissini	*Hyotissa hyotis* (Linnaeus, 1758)	Type (as *Mytilus hyotis*) of *Hyotissa* Stenzel, 1971	Florida Keys, FK-717	FMNH 302010	AY548883
	Hyotissa hyotis (Linnaeus, 1758)	Same as previous	Guam	UMMZ 265995	AY376599
	Hyotissa mcgintyi (Harry, 1985)	New name for *Ostrea thomasi* McLean, 1941; type of *Parahyotissa* Harry, 1985	Florida Keys, FK-650 and FK-717	UMMZ 300092 (for DNA), FMNH 302057, AMNH 308091	AY376597
	Hyotissa numisma (Lamarck, 1819)	Type (as *Ostrea numisma*) of *Parahyotissa (Numismoida)* Harry, 1985	Guam	UMMZ 265996	AY376598
Pycnodonteinae: Neopycnodontini	*Neopycnodonte cochlear* (Poli, 1795)	Type (as *Ostrea cochlear*) of *Neopycnodonte* Stenzel, 1971	Maui, Hawaii	UMMZ 265997	AY376600

```
                  1                                                                                                   100
H. hyotis Guam    CGCCACTCGATTGTCAYGTGTGGTAGTACCTGCCCAGTGCGCGAGATGCGTAAACGGYGGCTGTAACCTGACGGTCCTAAGGTAGCGAAATTCCTAGCCC
H. hyotis Florida ----------------C----------------------------------------T---------------------------------------

                  101                                                                                                 200
H. hyotis Guam    TTTAATTGGGGGCCTGCATGAAGGTTTGACGAGGGCTTCACTGTCTCTTGCCTTGAGAGATTGAAATTTAGTTAGGGGTGCAGACGCCCTTGTAGTAAAG
H. hyotis Florida ----------------------------------------------------------------------------------------------------

                  201                                                                                                 300
H. hyotis Guam    TTAGACAAAAAGACCCCGTGCAACTTTAAAGAGGGGTGCTGGTATTTGAACTCGTCTTATTTTTTGGTGGGGCGCCAGGGAAGTATACAAAACCTTCTCT
H. hyotis Florida ----------------------------------------------------------------------------------------------------

                  301                                                                                                 400
H. hyotis Guam    TATAGAAACAACGCGACACGTGATGATCCAAATTTGTTTGAGAATAGGAGAAGTTACGCCGGGGATAACAGAGCAATCCTGCAGTAGAGTCCGTATTAGC
H. hyotis Florida ----------------------------------------------------------------------------------------------------

                  401                                                                                           492
H. hyotis Guam    TGCGGGGATTGCCACCTCGATGTTGAATTATGGATGATAACTCTTTGGTTGCAGCAGCTATTGAAGTAGGTCTGTTCGACCTTTGAAACCTT
H. hyotis Florida ------------------------------------------------------------------------------------------------
```

Fig. 5. Alignment of two *Hyotissa hyotis* mitochondrial large ribosomal subunit (16S) gene fragments. The top sequence was obtained from a western Pacific specimen sampled in Guam (Kirkendale *et al*. 2004) and the bottom sequence is from a western Atlantic specimen sampled in the Florida Keys. Dashes in the latter indicate nucleotide identity to the Guam specimen. Note that in the two positions that vary among the samples, the Guam specimen exhibited a C/T heteroplasmic condition, here represented by a 'Y'.

outgroup (Ó Foighil and Taylor 2000). Analyses were performed using the heuristic search option with 100 random stepwise additions and tree bisection-reconnection (TBR) branch-swapping. Characters were unordered and equally weighted, and inferred sequence gaps were considered as missing data. Branch support levels were estimated with bootstrapping (Felsenstein 1985) (1000 replications, heuristic searches, 10 random additions each).

Institutional acronyms

AMNH	American Museum of Natural History, New York, New York, USA
ANSP	Academy of Natural Sciences of Philadelphia, Pennsylvania, USA
FMNH	Field Museum of Natural History, Chicago, Illinois, USA
UMMZ	Museum of Zoology, University of Michigan, Ann Arbor, Michigan, USA

Results and discussion

Fully grown specimens of *Hyotissa hyotis* are relatively easy to separate from those of *H. mcgintyi*, by simple virtue of size: *H. hyotis* attains a shell length nearly twice that of *H. mcgintyi* (Table 2). The other obvious difference is the dark shell colouration (black in the Florida specimen, black or brown in the Indo-Pacific material), and the black body colouration of *H. hyotis*, both of which are much lighter in *H. mcgintyi*. Internally, shell colouration varies somewhat, but *H. hyotis* was consistently darker closer to the shell margin, while *H. mcgintyi* was consistently lighter and never displayed any black or dark-brown pigment. Both species are irregularly sculptured externally, can have wavy, saw-toothed margins, and of course have the vesicular shell structure characteristic of Gryphaeidae (distinguishing them from other saw-toothed oysters, e.g. *Dendostrea frons* (Linnaeus, 1758), *Ostreola equestris* (Say, 1834)). No juvenile specimens of *H. hyotis* have been available in this study. Thomson (1954) described small Indo-Pacific specimens of what he thought to be *H. hyotis* as having very shallow lower valves, weakly crenulated margins, reddish purple radial lines on the external shell margin, and chalky white or greenish shell interior. However, it must be noted that these supposed *H. hyotis* juveniles were assignable to the nominal species *Ostrea procles* Iredale, 1939, which Thomson (1954: 161) considered synonymous with *H. hyotis*. *Ostrea procles* is now considered a synonym of *Hyotissa numisma* (Lamarck, 1819) (e.g. Lamprell and Healey 1998).

The Floridian specimen of *Hyotissa hyotis* had an almost identical 16S mitochondrial genotype to that obtained from a western Pacific (Guam) conspecific by Kirkendale *et al*. (2004), differing in only two nucleotide positions (Fig. 6). Interestingly, both variable positions were heteroplasmic in the Guam specimen: two nucleotide peaks (C/T) in the same position on both DNA strands. The Floridian specimen had a clean C and a clean T

Table 2. Comparison of shell features of Florida *Hyotissa* species

	Max. shell diameter	Margin	External colouration	Internal colouration
Hyotissa hyotis	To 18 cm (single specimen)	Wavy, large saw-toothed	Purplish black	Dirty bluish-white in centre to bluish black toward margin (reminiscent of *Pinctada margaritifera* (Linnaeus, 1758))
Hyotissa mcgintyi	To 10 cm	Irregular or saw-toothed	Cream, pinkish or lavender	Cream, pinkish, dirty light brown; in fresh specimens lightest coloured area often near shell margin

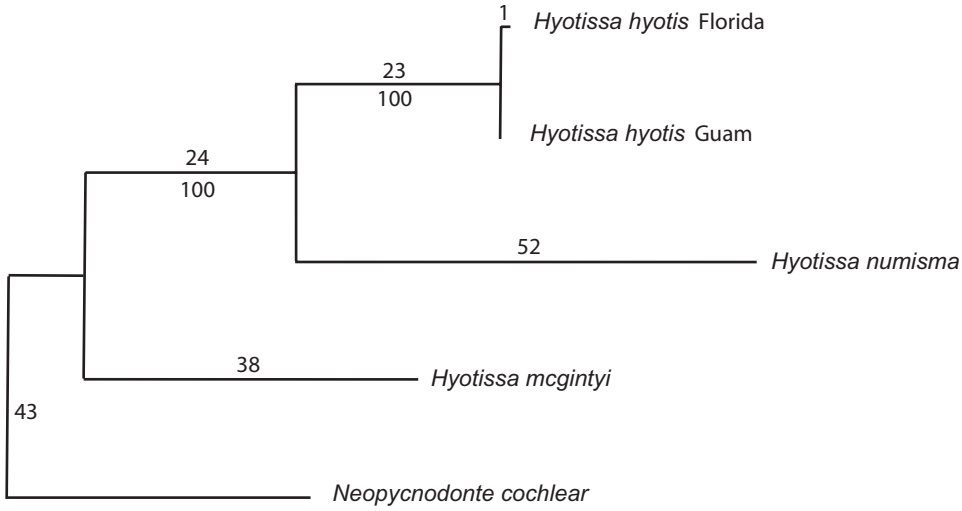


Fig. 6. Most parsimonious tree (173 steps, *CI* = 0.948, *RI* = 0.824) obtained by an exhaustive search for optimal trees (PAUP*) of available gryphaeid mitochondrial 16S genotypes (Table 1). *Neopycnodonte cochlear* was the designated outgroup, characters were unordered and equally weighted, and inferred sequence gaps were considered as missing data. Respective number of steps is indicated above each branch and the bootstrap values (Felsenstein 1985) supporting each node are presented below the branches.

in homologous sites. These ambiguous positions in the Guam specimen were coded as 'Y' (pyramidine) in our analyses and it appears that this *H. hyotis* individual contained multiple mitochondrial 16S genotypes, one of which could be identical to that of the homoplasmic Floridian conspecific. Mitochondrial heteroplasmy, in the form of Doubly Uniparental Inheritance (DUI; Zouros *et al*. 1994) systems, has been documented in several distinct bivalve clades (Skibinski *et al*. 1994; Hoeh *et al*. 1996; Liu *et al*. 1996; Passamonti and Scali 2001). To our knowledge, DUI has yet to be demonstrated in oysters, so the significance of the apparent mitochondrial heteroplasmy in the Guam *H. hyotis* individual remains to be established. Phylogenetic analysis of the gryphaeid mitochondrial 16S dataset placed the Floridian *H. hyotis* specimen in a robust, shallow tip clade with its Guam conspecific (Fig. 4) and unambiguously corroborated its initial taxonomic identification based on conchological features.

Neither *Hyotissa* species appears to be common in south-eastern Florida waters, (probably due to the lack of suitable 'hard-bottom' substrata, apart from shipwrecks), but *H. mcgyntyi* has well established regional populations in the Gulf of Mexico. It is 'abundant on the offshore coral reefs off Texas and locally almost reef forming on Miocene shale outcrops' according to Odé (1980: 49, as *Pycnodonte hyotis*), and has been collected since the 1960s (with the popularisation of scuba diving) from deeper reefs, such as the East and West Flower Gardens off the coasts of Texas and Louisiana (Harry 1986*b*). In other areas, artificial hard-bottom seems to provide an attractive opportunity for *H. mcgintyi* settlement: according to Harry (1986*b*: 16), it 'is one of the most abundant oysters on offshore oil platforms, of which there are literally thousands in the waters, chiefly off Louisiana'. In sharp contrast, we are aware of but two reliable records of *H. hyotis* in the western Atlantic, both very recent and restricted to Florida: this present finding and another adult shell retrieved in 30 m depth off West Palm Beach in 2001 (G. Paulay, personal communication).

The exceptionally large size of *H. hyotis* implies that this species is unlikely to have been underrepresented in historical regional biotic surveys. Its discovery therefore most likely stems from a recent, previously undocumented invasion of the south-eastern USA coastline by Indo-Pacific taxa, as has lately occurred in the case of the black-lipped pearl oyster *Pinctada margaritifera* (Linnaeus, 1758) (Chesler 1994; Carlton 1996; M. Bukstel personal communication; R. D. Shearer personal communication) and more conspicuously the green mussel *Perna viridis* (Linnaeus, 1758) (via Trinidad; Benson *et al*. 2001; Ingrao *et al*. 2001). Transport via the international shipping industry, as part of fouling and/or bilge water fauna, has been implicated in these latter cases, and is likely involved in the case of *H. hyotis* as well.

Acknowledgments

This is a result of the Florida Keys Molluscan Diversity Project, funded in part by a grant of the Comer Research and Education Foundation to RB and PMM, as well as Field Museum's Women's Board, and was conducted under Florida Keys National Marine Sanctuary permit 2002–078 and accompanying Florida saltwater fishing licenses. Laboratory research was supported by NSF awards DEB/PEET-9978119 to RB and PMM and by OCE-0099084 to DÓF. Gustav Paulay (University of Florida, Gainesville) kindly provided information on the other Florida *Hyotissa hyotis* sample and also donated the Guam conspecific employed in the earlier study cited herein. Gary Rosenberg (ANSP) furnished information on *Hyotissa* holdings at ANSP; Luiz Ricardo L. Simone (Museu de Zoologia da Universidade de São Paulo) helped us locate a copy of Celso Guimarães Prado's article. Robert D. Shearer and Mary Bukstel (Boca Raton, Florida) provided data on confirmed live-collected specimens of *Pinctada margaritifera* recently taken by scuba divers off eastern Florida.

References

Abbott, R. T. (1974). 'American Seashells: the Marine Mollusca of the Atlantic and Pacific Coasts of North America.' 2nd edn. (Van Nostrand Reinhold: New York, USA.)

Benson, A. J., Marelli, D. C., Frischer, M. E., Danforth, J. M., and Williams, J. D. (2001). Establishment of the green mussel, *Perna viridis* (Linnaeus, 1758) (Mollusca: Mytilidae) on the west coast of Florida. *Journal of Shellfish Research* **20**, 21–29.

Carlton, J. T. (1996). Marine bioinvasions: the alteration of marine ecosystems by nonindigenous species. *Oceanography* **9**, 36–43.

Carriker, M. R., and Gaffney, P. M. (1996). A catalogue of selected species of living oysters (Ostreacea) of the world. In 'The Eastern Oyster'. (Eds V. S. Kennedy, R. I. E. Newell and A. F. Eble.) pp. 1–8. (Maryland Sea Grant College: College Park, MD, USA.)

Celso Guimarães Prado, A. (1996). Occurrence of *Pycnodonte hyotis* (Linnaeus, 1758), (Gryphaeidae, Pycnodontinae) for brazilian [sic] coast (Maranhão State). *Publicações Ocasionais Conquiliologistas do Brasil* **1995**, 12.

Chesler, J. (1994). Not just bilge water. *American Conchologist* **22**, 13.

Espinosa, J., and Juarrero, A. (1989). Moluscos bivalvos del litoral rocoso de Ciudad Habana. *Revista de Investigaciones Marinas* **10**, 125–132.

Espinosa, J., Ortea, J., and Valdés, Á. (1994). Catalog de los moluscos bivalvos recientes del Archipiélago cubano – Catalogue of the recent bivals [sic] molluscs from the cuban [sic] Archipelago. *Avicennia* **2**, 109–129.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Glenn, L. C. (1904). Systematic paleontology of the Miocene deposits of Maryland: Pelecypoda. In 'Maryland Geological Survey'. (Ed. W. B. Clark.) pp. 274–401, pls 65–108. (Johns Hopkins Press: Baltimore, MD, USA.)

Harry, H. W. (1985). Synopsis of the supraspecific classification of living oysters (Bivalvia: Gryphaeidae and Ostreidae). *The Veliger* **28**, 121–158.

Harry, H. W. (1986*a*). Sententia: The relevancy of the generic concept to the geographic distribution of living oysters (Gryphaeidae and Ostreidae). *American Malacological Bulletin* **4**, 157–162.

Harry, H. W. (1986*b*). Oysters of the northwestern Gulf of Mexico. *Texas Conchologist* **23**, 14–19.

Hoeh, W. R., Stewart, D. T., Sutherland, B. W., and Zouros, E. (1996). Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). *Evolution* **50**, 2276–2286.

Ingrao, D. A., Mikkelsen, P. M., and Hicks, D. W. (2001). Another introduced marine mollusk in the Gulf of Mexico: The Indo-Pacific green mussel, *Perna viridis*, in Tampa Bay, Florida. *Journal of Shellfish Research* **20**, 13–19.

Kessing, B., Croom, H., Martin, A., McIntosh, C., McMillan, W. O., and Palumbi, S. (1989). 'The Simple Fool's Guide to PCR.' (University of Hawaii: Honolulu, HI, USA.)

Kirkendale, L., Lee, T., and Baker, P. and Ó Foighil, D. (2004). Oysters of the Conch Republic (Florida Keys); a molecular phylogenetic study of *Parahyotissa mcgintyi, Teskeyostrea weberi* and *Ostreola equestris*. In 'Bivalve Studies in the Florida Keys, Proceedings of the International Marine Bivalve Workshop, Long Key, Florida, July 2002'. (Eds R. Bieler and P. M. Mikkelsen.) Published as *Malacologia* **46**, 309–326.

Lamprell, K., and Healey, J. (1998). 'Bivalves of Australia Volume 2.' (Backhuys Publishers: Leiden, The Netherlands.)

Liu, H., Mitton, J. B., and Wu, S. (1996). Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the freshwater mussel, *Anodonta grandis grandis. Evolution* **50**, 952–957.

McLean, R. A. (1941). The oysters of the western Atlantic. *Notula Naturae* **67**, 1–14.

Mikkelsen, P. M., and Bieler, R. (2000). Marine bivalves of the Florida Keys: discovered biodiversity. In 'The Evolutionary Biology of the Bivalvia'. (Eds E. M. Harper, J. D. Taylor and J. A. Crame.) Published as *Geological Society, London, Special Publications* **177**, 367–387.

Odé, H. (1980). Distribution and records of the marine Mollusca in the northwest Gulf of Mexico. *Texas Conchologist* **16**, 44–52.

Ó Foighil, D., and Taylor, D. J. (2000). Evolution of parental care and ovulation behavior in oysters. *Molecular Phylogenetics and Evolution* **15,** 301–313.

Palumbi, S. R. (1996). Nucleic Acids II: the polymerase chain reaction. In 'Molecular Systematics'. 2nd edn. (Eds D. M.Hillis, C. Moritz and B. K. Mable.) pp. 205–247. (Sinauer Associates, Inc.: Sunderland, MA, USA.)

Passamonti, M., and Scali, V. (2001). Gender-associated mitochondrial DNA heteroplasmy in the venerid clam *Tapes philippinarum* (Mollusca: Bivalvia). *Current Genetics* **39**, 117–124. doi:10.1007/S002940100188

Ranson, G. (1941). Les espèces actuelles et fossiles du genre *Pycnodonta* F. de W. – I. *Pycnodonta hyotis* L. *Bulletin du Muséum National d'Histoire Naturelle, Paris* **13**, 82–92.

Ranson, G. (1967*a*). Les espèces d'huîtres vivant actuellement dans le monde defines par leurs coquilles larvaires ou prodissoconques. Étude des collections de quelques-uns des grands musées d'histoire naturelle. *Revue des Travaux de l'Institut des Peches Maritimes, Paris*, **31**, 127–192.

Ranson, G. (1967*b*). Les espèces d'huîtres vivant actuellement dans le monde defines par leurs coquilles larvaires ou prodissoconques. Étude des collections de quelques-uns des grands musées d'histoire naturelle (suite). *Revue des Travaux de l'Institut des Peches Maritimes, Paris*, **31**, 205–247.

Rosenberg, G. (1992). 'The Encyclopedia of Seashells.' (Dorsett Press: New York, NY, USA.)

Saville-Kent, W. (1893). 'Great Barrier Reef of Australia: its Products and Potentialities.' (W. H. Allen & Company: London, UK.)

Sevilla-H., M. L., Garcia-D., F., and Uria-G., E. (1998). Datos anatomicos de *Hyotissa hyotis* (Linnaeus, 1758), Ostreacea: Gryphaeidae. *Anales de la Escuela Nacional de Ciencias Biológicas, México* **43**, 25–32.

Skibinski, D. O. F., Gallagher, C., and Benyon, C. M. (1994). Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis. Genetics* **138**, 801–809.

Stenzel, H. B. (1971). Oysters. In 'Treatise on Invertebrate Paleontology, Part N, Mollusca 6, Bivalvia'. Vol. 3. (Ed. R. C. Moore.) pp. N953–N1224. (Geological Society of America and University of Kansas: Lawrence, KS, USA.)

Swofford, D. L. (2002). 'PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).' Version 4.0. (Sinauer Associates: Sunderland, MA, USA.)

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). The CLUSTAL_X window interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4882. doi:10.1093/NAR/25.24.4876

Thomson, J. M. (1954). The genera of oysters and the Australian species. *Australian Journal of Marine and Freshwater Research* **5**, 132–168.

Torigoe, K. (1981). Oysters in Japan. *Journal of Science of the Hiroshima University Series B Division 1 (Zoology)* **29**, 291–419.

Turgeon, D. D., Quinn, J. F. Jr, Bogan, A. E., Coan, E. V., Hochberg, F. G., Lyons, W. G., Mikkelsen, P. M., Neves, R. J., Roper, C. F. E., Rosenberg, G. Roth, Scheltema, A., Thompson, F. G., Vecchione, M., and Williams, J. D. (1998). 'Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Mollusks, 2nd ed.' (Bethesda, Maryland.) Published as *American Fisheries Society, Special Publication* **26**, 1–526.

Zouros, E., Oberhauser Ball, A., Saavedra, C., and Freeman, K. R. (1994). An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus. Proceedings of the National Academy of Sciences of the United States of America* **91**, 7463–7467.