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Sympatric Australian *Lasaea* species (Mollusca: Bivalvia) differ in their ploidy levels, reproductive modes and developmental modes

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The cosmopolitan marine bivalve genus *Lasaea* is predominantly composed of highly polyploid asexual lineages with one exception: the diploid, sexual Australian species *L. australis*. Two undescribed, direct-developing congeners co-occur with the indirect-developing *L. australis* on the rocky intertidal of southeastern Australia. One of these, ***L. colmani* sp. nov.**, is also diploid and sexual. The other direct-developing congener is an asexual polyploid composed of a variety of clonal lineages. All three sympatric Australian *Lasaea* congeners are morphologically distinguishable, although prodissoconch distinctions are required to separate large polyploid clams from equivalently-sized *L. australis*. Similarities in mitochondrial gene sequence and in shell morphology suggest that *L. australis* and the Australian sympatric polyploid clones share an exclusive common ancestor despite differing in developmental mode, ploidy and reproductive mode. However, detailed karyological analyses failed to identify a chromosome set morphologically similar to that of *L. australis* among the sympatric Australian polypoid complement. We propose that generation of the polyploid Australian clones (presumably by hybridization) was followed by radical karyological rearrangement.

ADDITIONAL KEYWORDS:—karyology – polyploid – asexual – clam – marine – hybrid – ideogram.

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INTRODUCTION

Polyploidization has been remarkably influential in the evolution of plants and much of our understanding of the dynamics of genome duplication stems from botanical research (Soltis & Soltis, 1993, 1995). Although much less common in animals (Orr, 1990), some taxa, such as several subgenera of freshwater pulmonate snails, are predominantly polyploid (Burch & Huber, 1966; Goldman & LoVerde, 1983; Städler, Loew & Streit, 1993, 1995). Recent application of molecular techniques to polyploid taxa has greatly increased our understanding of their evolutionary origins (Soltis & Soltis, 1993). As defined by Thompson & Lumaret (1992), genome duplication may: (1) occur within a species (autopolyploidy); (2) result from hybridization among ancestral species that were partially cross-fertile (segmental allopolyploids); (3) result from hybridization among ancestral species that were almost completely cross-sterile (genomic allopolyploids). The latter case is the one most frequently encountered in natural populations (Soltis & Soltis, 1993). Detailed genetic analyses of synthetic and natural allopolyploid plant taxa have found that hybrid genomes may undergo rapid and dramatic rearrangements prior to stabilization (Bennett, Kenton & Bennett, 1992; Kenton *et al.*, 1993; Song *et al.*, 1995).

Few plant or animal taxa match the pronounced levels of genome duplication recorded for asexual polyploid lineages of the cosmopolitan marine clam genus *Lasaea* (Thiriot-Quiévreux *et al.*, 1988, Thiriot-Quiévreux, Insua Pombo & Albert, 1989; Ó Foighil & Thiriot-Quiévreux, 1991). These minute, hermaphroditic, crevice-dwelling bivalves occur on most rocky shores worldwide (Beauchamp, 1986; Ó Foighil, 1989). Apart from *Lasaea australis* (Lamarck, 1818), karyologically-characterized *Lasaea* populations (from the northeastern Pacific, northeastern Atlantic, Mediterranean, and the southern Indian Ocean) are composed of highly polyploid clonal lineages, many of which display supernumerary chromosomes (Ó Foighil, 1988; Tyler-Walters & Crisp, 1989; Thiriot-Quiévreux, 1992; Thiriot-Quiévreux *et al.*, 1988, 1989; Ó Foighil & Thiriot-Quiévreux, 1991; Thiriot-Quiévreux, unpubl.). Intensive karyological studies have failed to find meiotic metaphases in the polyploid clones (Thiriot-Quiévreux *et al.*, 1988, 1989; Ó Foighil & Thiriot-Quiévreux, 1991) and, at least in northeastern Pacific clones, eggs are activated gynogenetically by autosperm (Ó Foighil & Thiriot-Quiévreux, 1991). Expression of non-segregating fixed heterozygous allozyme phenotypes by *Lasaea* clones (Crisp *et al.*, 1983; Ó Foighil & Eernisse, 1988; Crisp & Standen, 1988; Tyler-Walters & Crisp, 1989; Taylor & Ó Foighil, 2000) is consistent with a genomic allopolyploid origin for these asexual lineages, although Tyler-Walters & Hawkins (1995) recently presented preliminary evidence for parent/progeny differences in a RAPD marker. Despite

lacking dispersive larvae, these asexual organisms have collectively attained a remarkably extensive geographic range, achieved possibly by rafting (Ó Foighil, 1989).

Molecular phylogenetic analyses indicate that Australian members of *Lasaea* may be central to understanding the evolutionary history of this cosmopolitan genus (Ó Foighil & Smith, 1995, 1996). One of these, *L. australis*, is plausibly be the most numerous mollusc on southern Australian rocky shores and occurs in a wide variety of intertidal crevice microhabitats (Roberts, 1984; Polz, 1986; Tong, 1990). This species is diploid ($2n=36$), meiotic (Thiriot-Quiévreux, 1992), and exhibits segegrating allozyme alleles that largely conform to Hardy–Weinberg expectations (Ó Foighil, 1988; Tyler-Walters & Crisp, 1989). *L. australis* is an indirect-developer, releasing its brood as planktotrophic straight-hinged veliger larvae (Ó Foighil, 1988; Tyler-Walters & Crisp, 1989). Unidentified direct-developing *Lasaea* of unknown reproductive mode have also been reported from Australian rocky shores (Polz, 1986; Ó Foighil, 1989; Tong, 1990) and two undescribed, morphologically distinct, congeners occur sympatrically with *L. australis* in southeastern Australia (Ó Foighil & Smith, 1995). These three sympatric Australian congeners straddle a robust phylogenetic dichotomy evident in *Lasaea* mitochondrial gene trees (Ó Foighil & Smith, 1995, 1996) and may preserve clues to the origins and persistence of asexuality in this genus. In this present study we describe one of the Australian direct developers, *L. colmani* sp. nov. and contrast its morphology and karyology with that of its sympatric congeners.

MATERIALS AND METHODS

Sources of specimens

Samples of *Lasaea* were retrieved from three rocky intertidal sites in the Greater Sydney area, New South Wales, Australia during February 1992 (Fig. 1). In Clovelly, specimens were recovered from tide pool coralline algal cover and from clumps of the tubiculous polychaete *Galeolaria caespitosa* (Savigny, 1818) at the upper limit of its distribution. At the North Harbour site, sampling efforts focused on oyster clump interstices at the upper limit of *Saccostrea commercialis* (Iredale & Roughley, 1933) distribution. At the Long Reef headland, *Galeolaria caespitosa* aggregations were sampled at the mid zone of this species intertidal distribution. All specimens of *Lasaea* were removed from the sampled substrate, segregated according to morphotype, and kept alive in room temperature seawater until processed further.

Shell morphologies of air-dried specimens of Australian *Lasaea* were documented using a Wild Photomacroscope and a JOEL JSM-35 scanning electron microscope. Brooded embryos were dissected from the suprabranchial chambers of *L. colmani* sp. nov., and of sympatric Australian polyploid congeners, and photographed with a Nikon Optiphot light microscope.

Karyology

Specimens of *L. colmani* sp. nov. ($n=30$), and of the Australian polyploid direct developer ($n=40$), were airmailed live to Villefranche-sur-Mer. They were maintained in aquaria and fed cultured microalgae for 9 days to stimulate cell division.

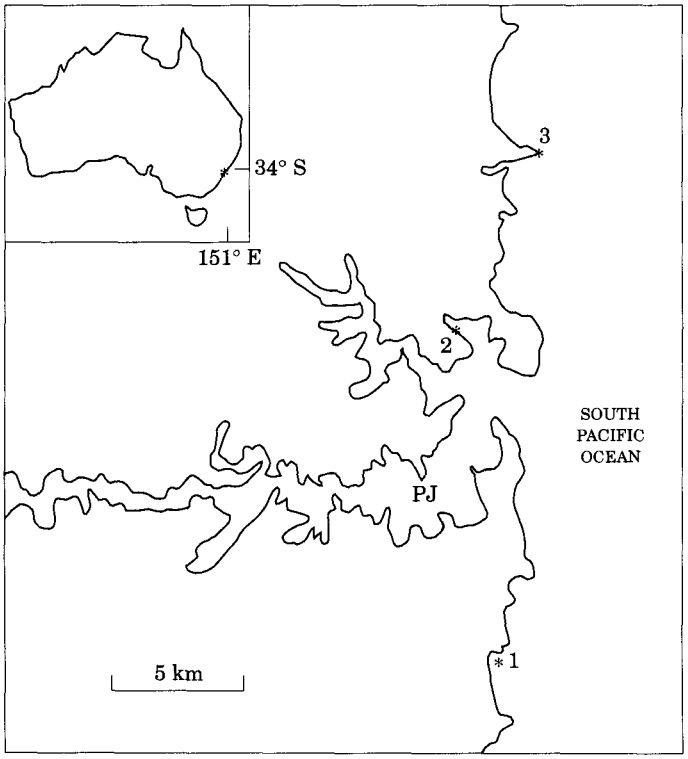


Figure 1. Map of the Sydney area shoreline showing the *Lasaea* spp. sampling sites. The insert indicates Sydney's location (asterisked) in southeastern Australia. The main map gives the location of Port Jackson (PJ) for spatial reference and the sampling sites are numbered 1–3: (1) Clovelly; (2) North Harbor; (3) Long Reef.

Specimens were then incubated for 12 hours in aerated seawater containing 0.005% colchicine and subsequently their valves were gently parted to allow effective hypotonic treatment (50 minutes in 0.9% sodium citrate). Further processing involved fixation in freshly mixed absolute alcohol and acetic acid (3:1) with three changes of 20 minutes duration. During the second fixation step, bodies were dissected out and slide preparations were made from one or two bodies of a particular species using an air drying technique (Thiriot-Quiévreux & Ayraud, 1982). The preparations were stained for 10 minutes with 4% Giemsa (pH 6.8) and photographs of well-spread metaphases were taken with a Zeiss III photomicroscope. For karyotyping, chromosomes were cut out of photomicrographs and were paired on the basis of size and centromere position. Measurements of chromosomes were made with a digitizer table (Summa Sketch III) interfaced to a Macintosh computer. Means of the relative length and centromeric indices were calculated for each chromosome pair (Thiriot-Quiévreux, 1992).

Allozyme electrophoresis

A subsample of live Australian polyploid direct developers ($n = 60$) was transported live to North America for allozyme analyses using standard 13% starch gel electrophoresis techniques as previously described (Ó Foighil, 1988). The aim was to look

TABLE 1. Relative frequencies of the three Australian *Lasaea* congeners obtained from 300 cm^2 of cover from Sydney area sampling locations

Location	No. *L. australis*	No. ***L. colmani* sp. nov.**	No. polyploids
Clovelly, tide pool coralline algae	285	0	0
Clovelly, upper limit of *Galeolaria caespitosa*	248	3	0
North Harbor, upper limit of *Saccostrea commercialis*	66	0	85
Long Reef, mid zone of *Galeolaria caespitosa* distribution	350	41	1

for evidence of allelic segregation in polymorphic loci in the polyploid direct developer. The following enzymes were assayed: phosphoglucomutase, mannose-6-phosphate isomerase, malate dehydrogenase and glucose-6-phosphate-isomerase (GPI; EC 5.3.1.9). GPI produced three distinct alleles and all 60 individuals were typed for this locus using the scoring method of Tracey *et al.* (1975).

Lamarck's (1818) syntypes of *Lasaea australis* (type locality = Timor), and the specimens he designated as "var. 2" of this species from "Port du Roi Georges, Nouvelle Holland" [presumably King George Harbor, Western Australia (Laseron, 1956)], were obtained on loan from the Muséum National d'Histoire Naturelle, Paris. Holotypes of *Lasaea rubra hinemoa* Finlay, 1928 and of *Lasaea maoria* (Powell, 1933) were obtained from the Auckland Museum.

A concise species description together with the designated type locality and museum registration numbers for type specimens of *L. colmani* sp. nov. is presented in the Appendix.

RESULTS

Table 1 summarizes the relative abundance of the three sympatric Australian *Lasaea* congeners in the various sampling locations. *L. australis* was present in large numbers in all samples, being the sole *Lasaea* species encountered in tide pool coralline algal cover, and being numerically dominant in all locations except at the upper limit of *Saccostrea commercialis* crevice habitat in North Harbor. Polyploid congeners were common only in the latter habitat and, in this study, were rarely encountered lower in the intertidal zone, although in some sites they may be moderately abundant there (W. Ponder, pers. comm.). *L. colmani* sp. nov. occurred only in samples taken from *Galeolaria caespitosa* aggregations, being rare in the Clovelly sample taken at the upper limit of the tubiculous polychaete cover and moderately common in Long Reef samples from the middle of the *G. caepitosa* zone.

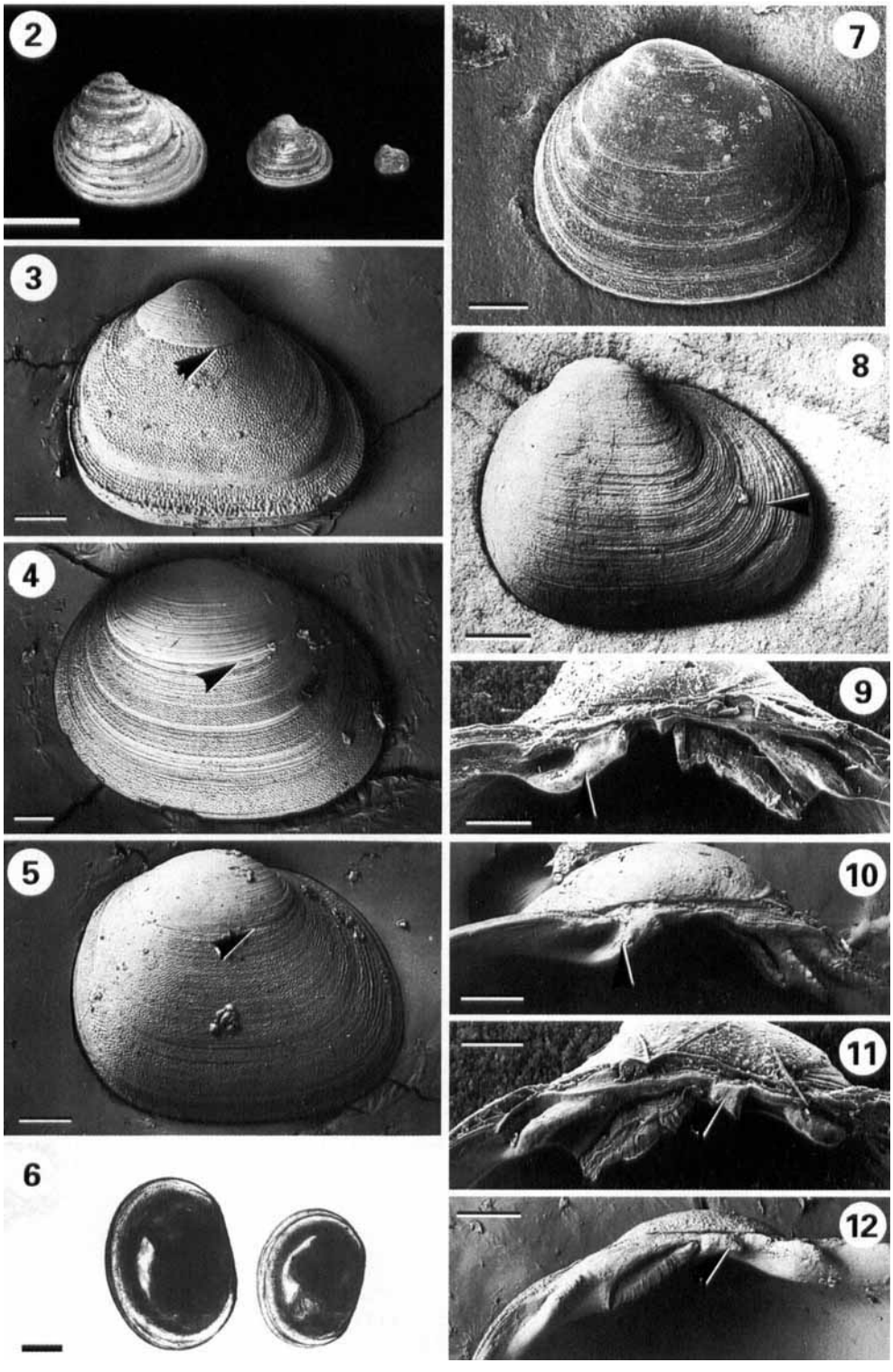
Shell morphology

Figure 2 shows the general appearances and relative adult sizes of the three sympatric Australian *Lasaea* congeners encountered in this study. *L. australis* was the largest of the three, reaching a maximum length of 6.5 mm. This compares to maximum encountered valve lengths of 3.7 mm for the polyploid direct developer and 1.4 mm for *L. colmani* sp. nov. Juveniles of the three sympatric congeners are

easily distinguished based on the morphology of their respective prodissoconchs (prejuvenile shells). The distinctly umbonate prodissoconch of *L. australis* range from 220–300 μm in length (Fig. 3). Prodissoconchs of both direct developers (Figs 4, 5) are flattened, D-shaped and non-umbonate, but differ in their dimensions. The sympatric polyploid congener produces a distinctly larger prodissoconch, ranging in length from 420 to 480 μm. *L. colmani* sp. nov. prodissoconchs range from 300 to 340 μm in length, among the smallest recorded for direct developing *Lasaea* species (Ó Foighil, 1989; Iwasaki, 1996). This prodissoconch size distinction reflects the relative dimensions of the juveniles of each direct developing species upon release into the environment (Fig. 6). Prodissoconchs of the three Australian species are generally preserved on the adult shell throughout the lives of individual clams and may be used as convenient distinguishing morphological markers. Lamarck's syntypes of *Lasaea australis* from Timor and his "var. 2" specimens from "Port du Roi Georges, Nouvelle Holland" have an identical prodissoconch morphology to the Sydney study populations of *L. australis* and all share essentially the same conchological features in their adult shells. The morphological variation among Lamarck's material does not significantly differ from the considerable range of *L. australis* ecophenotypes present in Sydney populations, although the Timorese type material was larger, ranging up to 8 mm in valve length.

External shell sculpture of the three sympatric congeners consists of a background of microscopic pitting which is slightly more coarsely grained in *L. australis* (Fig. 3) than in the other two (Figs 4, 5). Individual *L. australis* frequently develop heavy concentric foldings on their external valve surfaces (Ponder, 1971; Roberts, 1984; Ó Foighil, 1988) as is evident in Figure 2. Many of the larger Australian polyploid specimens displayed a reduced form of this concentric folding (Figs 2, 7) but this feature is absent in *L. colmani* sp. nov. Larger specimens of *L. colmani* sp. nov. frequently develop a brown external periostracal layer, especially anteriorally, that is applied in concentric ridges over the shell surface (Fig. 8). Shell coloration varies

Figures 2–5. Fig. 2. Photomicrograph of the right valves of the three sympatric southeastern Australian *Lasaea* congeners showing the relative sizes of typical adult clams. From left to right: *L. australis*, asexual polyploid direct developer, and *L. colmani* sp. nov. Scale bar = 3 mm. Fig. 3. Scanning electron micrograph (SEM) showing the right valve of a juvenile *Lasaea australis*. Note the umbonate prodissoconch. Arrow indicates the prodissoconch/dissoconch boundary. Scale bar = 100 μm. Fig. 4. SEM. Right valve of a juvenile southeastern Australian polyploid direct developer. The prodissoconch is large, flattened and non-umbonate. Arrow indicates the prodissoconch/dissoconch boundary. Scale bar = 100 μm. Fig. 5. SEM. Right valve of a juvenile *L. colmani* sp. nov. The prodissoconch is non-umbonate, but smaller and less flattened than that of the sympatric polyploid direct developers. Arrow indicates the prodissoconch/dissoconch boundary. Scale bar = 100 μm. Fig. 6. Light photomicrograph of newly released juveniles of southeastern Australian polyploid direct developer (left) and of *L. colmani* sp. nov. (right). Scale bar = 100 μm. Fig. 7. SEM. Right valve of a mid-sized southeastern Australian polyploid direct developer. Scale bar = 250 μm. Fig. 8. SEM. Right valve of an adult *L. colmani* sp. nov. The entire umbonal region is inflated relative to that of sympatric congeners. Arrow indicates periostracal folds which are most obvious on the anterior of the valves. Scale bar = 250 μm. Fig. 9. SEM. Right hinge of a mid-sized southeastern Australian polyploid direct developer. Arrow indicates the anterior lateral tooth. Scale bar = 100 μm. Fig. 10. SEM. Right hinge of an adult *L. colmani* sp. nov. Arrow indicates the anterior lateral tooth. Scale bar = 100 μm. Fig. 11. SEM. Left hinge of a mid-sized southeastern Australian polyploid direct developer. Arrow indicates the small cardinal tooth. Scale bar = 100 μm. Fig. 12. SEM. Left hinge of an adult *L. colmani* sp. nov. Arrow indicates the vestigial cardinal tooth. Scale bar = 100 μm.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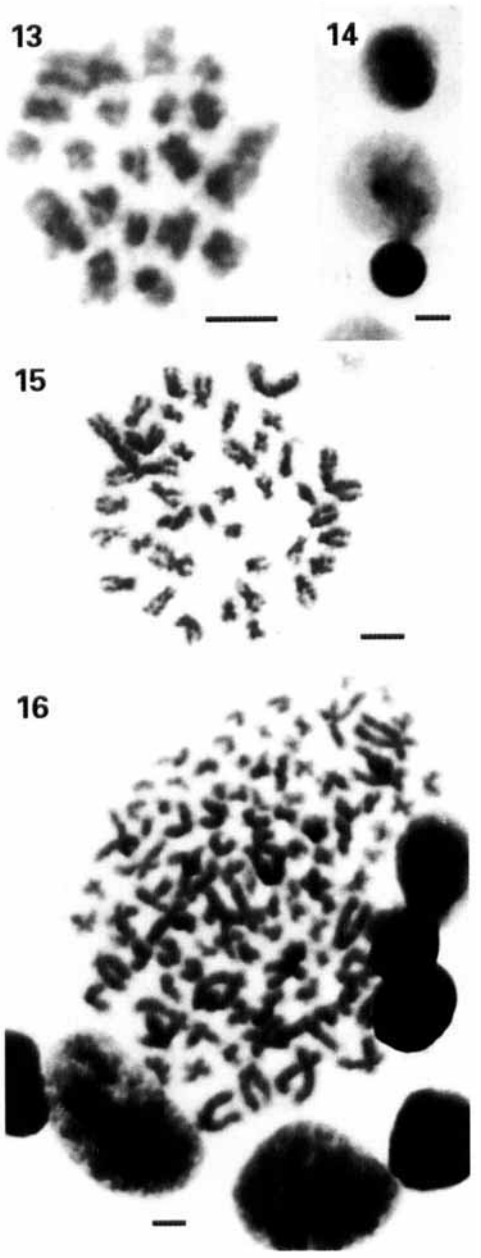
in all three species, from entirely white to entirely pinkish-red, with many individuals having abruptly changed shell colour during valve growth. Figures 7, 8 respectively show the valve outlines of a medium-sized polyploid direct developer and a full-sized *L. colmani* sp. nov. Note that the orthogyrate umbonal region of the latter is distinctly inflated, a feature more apparent in dorsal view, relative to the progyrate umbone of the polyploid congener. Other distinctions of *L. colmani* sp. nov. valves include a non-convex ventral margin, truncated posterior margin and a pronounced thinness and fragility. Specimens without a periostracal covering are transparent and it is difficult to avoid crushing their shells when manipulating individuals with a fine-tipped forceps. Figures 9–12 show the hinge structures of a polyploid direct developer and of *L. colmani* sp. nov. Both direct developers show the same hinge characters as *L. australis* (Ó Foighil, 1988), fully formed in the polyploids, but greatly reduced in prominence in *L. colmani* sp. nov. The right hinge (Figs 9, 10) has a short anterior lateral tooth, bearing a small spur on its posterior end, an oblique resilium and two lamellar posterior lateral teeth. A single truncated anterior lateral tooth is formed on the left hinge, together with a short thorn-like cardinal tooth, an oblique resilium and a lamellar posterior lateral tooth (Figs 11, 12). Adult *L. colmani* sp. nov. are easily distinguished from their sympatric congeners by their distinctive shell characters (see Appendix). It is much more difficult to distinguish larger polyploid clams from similarly-sized *L. australis* on the basis of gross shell morphology and examination of prodissoconch structure is often necessary to make a definitive identification.

Karyology

Out of 15 slide preparations of *L. colmani* sp. nov., 29 metaphases were scored for chromosome number. Ten displayed 40 chromosomes, nine showed 37–39 chromosomes and two exhibited a lower number. Chromosomes of the remaining 8 metaphases overlapped in their distributions and could not be scored accurately. A haploid number of $n=20$ was encountered in four meiotic cells (Fig. 13). Five metaphases (Fig. 15) were karyotyped and three metaphases were used for chromosome measurements. The *L. colmani* sp. nov. karyotype (Fig. 17) consists of 20 chromosome pairs, the first three being much larger than the others which show a gradual decrease in size. Five chromosome pairs are metacentric, nine are submetacentric, five are subtelocentric and one is telocentric (Fig. 19B).

The 48 slide preparations of the Australian polyploid direct developers examined in this study contained much larger interphase nuclei and mitotic metaphases (Fig. 16) than those of *L. colmani* sp. nov. (Figs 14, 15). No meiotic metaphases were encountered despite an intensive search for such. Thirty six mitotic metaphases were scored for chromosome number which ranged from 59 to 124 chromosomes. Eighteen metaphase spreads showed 90–100 chromosomes, ten had 111–124 chromosomes and eight had 59–89 chromosomes. Eight well-spread metaphases were karyotyped and four metaphases were used for chromosome measurements. The largest chromosomes clustered readily into subgroups of four on the basis of shared size and morphology. It was more difficult to so arrange the smallest chromosomes due to their less distinct morphologies, therefore some ambiguities remain. Figure 18 shows the karyotype generated from one mitotic metaphase with 94 chromosomes. Chromosome subgroupings were arranged in decreasing size according to the



Figures 13–15. Light photomicrographs of *L. colmani* sp. nov. Fig. 13. Meiotic metaphase ($n=20$). Fig. 14. Interphase nuclei. Fig. 15. Mitotic metaphase ($2n=40$). Fig. 16. Light photomicrograph showing the mitotic metaphase of a southeastern Australian polyploid direct developer (with 104 chromosomes) and interphase nuclei. Scale bars = 5 μm.

chromosome measurements of 18 tetrads derived from four metaphase cells. The polyploid karyotype consists of six metacentric, six submetacentric, five subtelocentric and one telocentric tetrad (Fig. 18). The remaining smaller chromosomes have been tentatively ordered in groups of four. Variation in chromosome number among

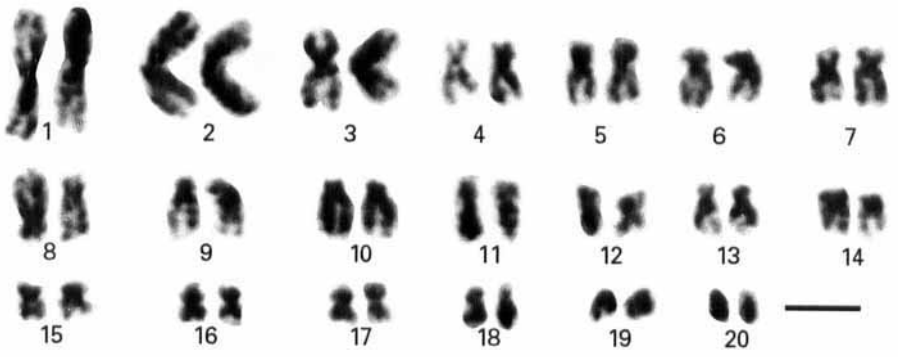


Figure 17. Karyotype of *L. colmani* sp. nov. ($2n = 40$). The chromosome pairs are arranged in decreasing size classes based on chromosome measurements taken from three metaphases. Scale bar = 5 μm.

metaphases resulted from the absence of a chromosome from individual subgroupings (e.g. subgroup #6 in Fig. 18) or from the occurrence of supernumerary subgroupings among the smallest chromosomes.

Ideograms were generated for both *L. colmani* sp. nov. and for the sympatric polyploid congener (largest 18 chromosomes) and are compared with that of *L. australis* (Thiriot-Quiévreux, 1992) in Figure 19. No concordance was evident between the chromosomal complements of either of the three sympatric Australian *Lasaea* congeners.

Allozyme analysis

Each of the 60 polyploid individuals typed for the GPI locus produced one of two electromorph patterns. Both patterns were composed of three evenly-spaced bands, the central band displaying the greatest staining intensity. This enzyme has a dimer subunit structure (Ó Foighil, 1988) and the electromorph patterns produced are consistent with a heterozygous condition in all of the animals typed. Three alleles were detected and the observed results differ markedly from random mating expectations (Table 2) and are consistent with the presence of two clonal lineages within the sample.

Brooding patterns

As is the case for all their studied congeners (Ó Foighil, 1985), both *L. colmani* sp. nov. and the sympatric polyploid congener are hermaphrodites and gonad squashes of mature ovotestes produced both sperm and eggs. Embryos are brooded in the suprabranchial chambers and brood size increased with adult size in both species. *L. colmani* sp. nov. brood sizes ranged from 3 to 12 embryos, occurring respectively in adults clams of 0.83 and 1.2 mm in valve lengths. Brood sizes in sympatric polyploids ranged from nine embryos in a 1.66 mm adult to 145 embryos in a 3.7 mm adult. Embryos of sympatric polyploid congeners were conspicuously larger than those of *L. colmani* sp. nov. at equivalent developmental stages, indicating greater parental investment/embryo in the former. Unlike its two sympatric congeners, the ctenidia of *L. colmani* sp. nov. lack outer demibranchs.

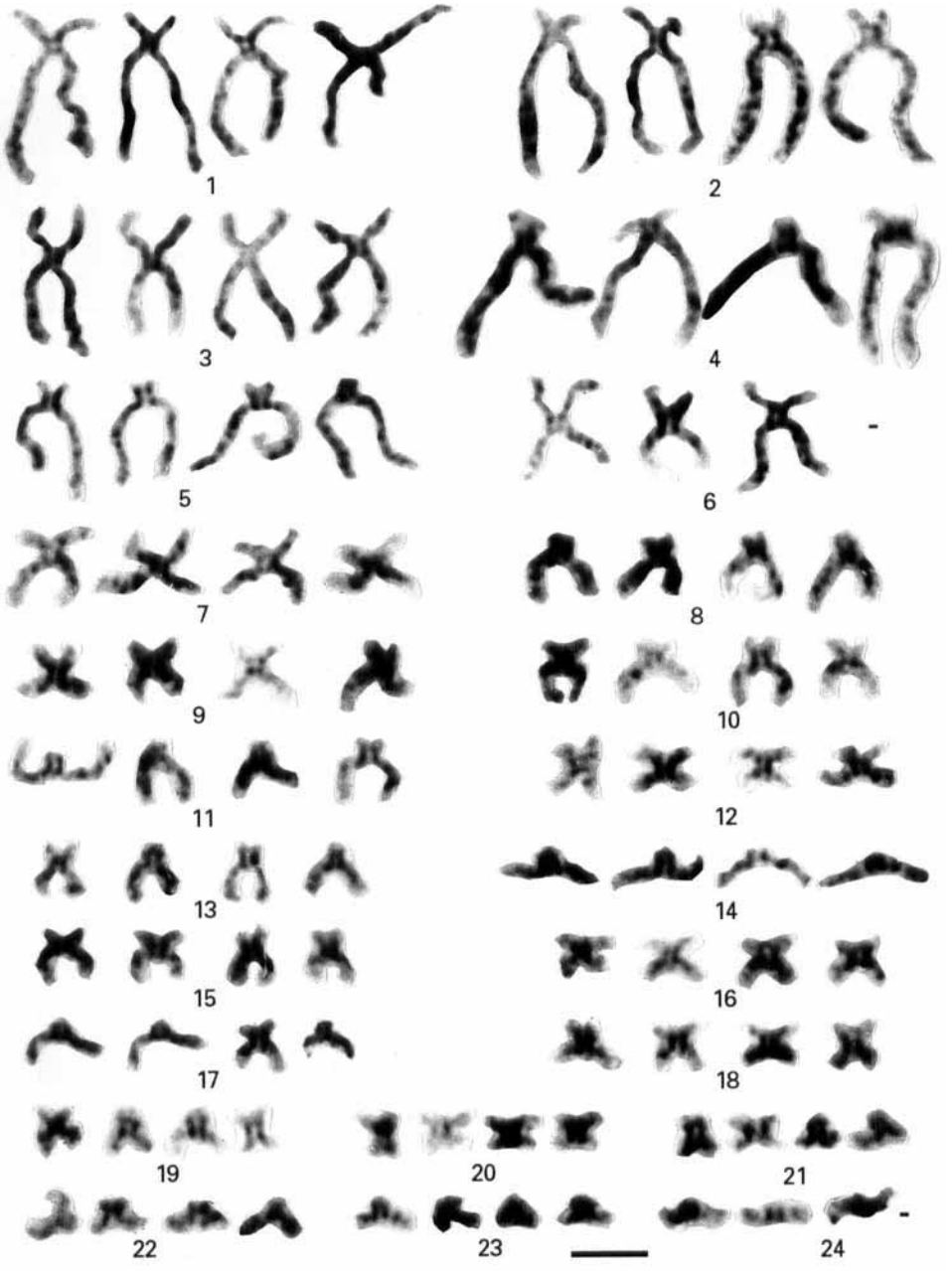


Figure 18. Karyotype of southeastern Australian polyploid direct developer (metaphase with 94 chromosomes). The chromosome quadrats are arranged in decreasing size classes based on chromosome measurements of the first 18 subgroupings taken from four metaphases. Scale bar = 5 μm.

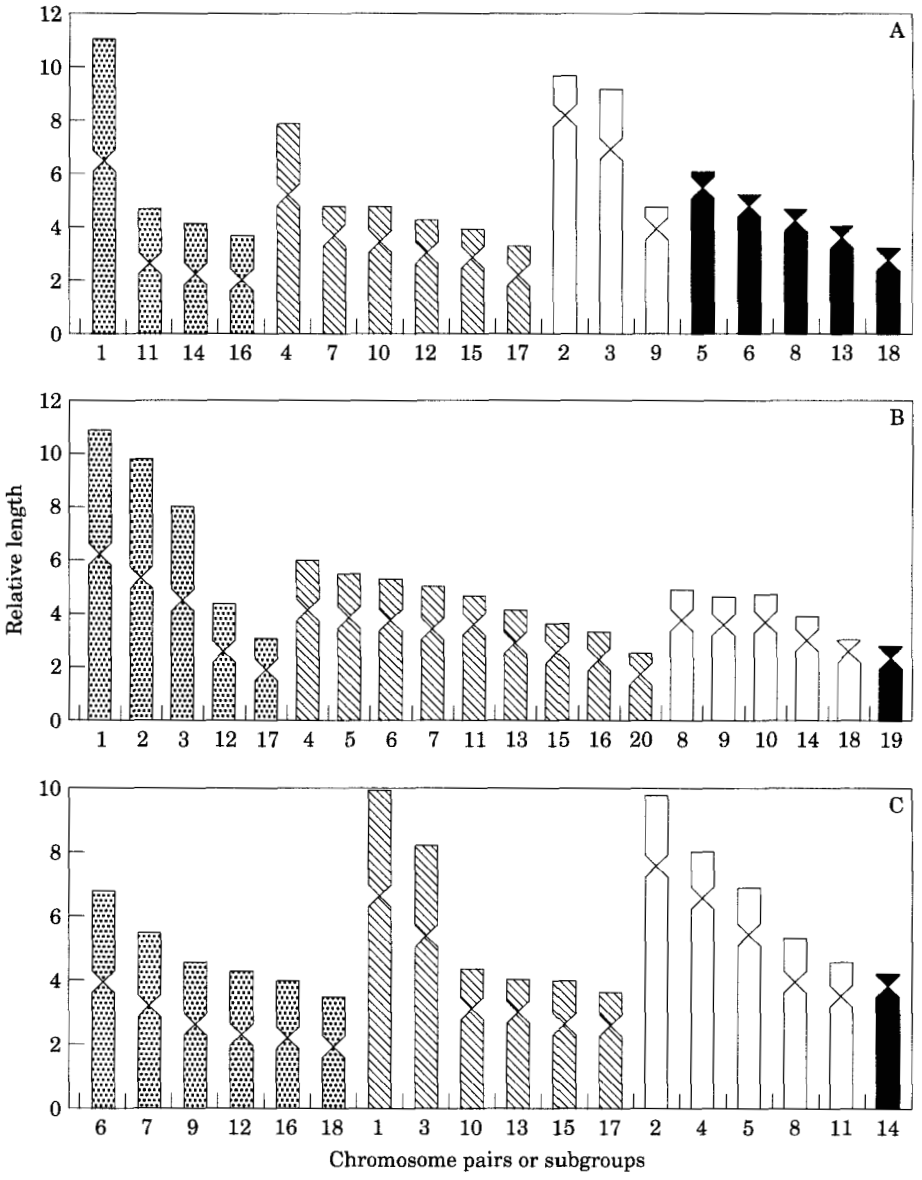


Figure 19. Ideograms constructed from the relative length and centromeric index values of (A) *Lasaea australis*, (B) *L. colmani* sp. nov., (C) southeastern Australian polyploid direct developer (the first 18 tetrads). (▨) metacentric; ▧ submetacentric; □ subtelocentric; ■ telocentric.

DISCUSSION

Sympatric Australian *Lasaea* congeners span the entire spectrum of developmental, reproductive and ploidy variation in this genus, unlike other karyologically-characterized *Lasaea* populations which are exclusively composed of direct-developing polyploid asexual clones (Thiriot-Quiévreux *et al.*, 1988, 1989; Ó Foighil & Thiriot-Quiévreux, 1991; Thiriot-Quiévreux, unpubl.). The discovery of *L. colmani* sp. nov.

TABLE 2. Genotype distributions of the three GPI alleles detected in 60 Australian polyploid direct-developing *Lasaea*. Allele 100 occurred in all 60 individuals; allele 89 was produced by 55 individuals and 5 individuals exhibited allele 95. Genotype distributions differ markedly from random mating expectations: the Chi-square value (54.203, df=3) shows significant deviation from Hardy–Weinberg–Castle equilibrium expectations ($P<0.0001$).

Genotype	Observed Frequency	H-W-C expected Frequency
89/89	0	12.479
89/95	0	2.311
89/100	55	27.731
95/95	0	0.084
95/100	5	2.521
100/100	0	14.874

is particularly interesting as this is the first record of a diploid sexual direct-developing species in the genus. Although *L. colmani* sp. nov. co-clusters with a subset of asexual congeners in mitochondrial gene trees, it is genetically very distinct and cannot be considered a parental species (Ó Foighil & Smith, 1995, 1996). *L. colmani* sp. nov. is also morphologically distinct from all of its direct-developing congeners (see Appendix) and has a much smaller adult size. It is possible, however, that additional diploid sexual direct developing congeners occur in the eastern Pacific. Further genetic characterization of direct developers throughout the global range of this taxon may yet uncover convincing parental species for at least some of the asexual lineages.

In some respects, the polyploid direct developer is the least remarkable of the three Australian congeners, being similar to other asexual *Lasaea* lineages in its direct development, pronounced polyploidy, absence of meiotic metaphases and generation of non-segregating heterozygous allozyme phenotypes. Of all the studied asexual lineages, however, the Australian polyploid clones exhibit the least mitochondrial genetic distance from one of the two known diploid sexual congeners, in this case *L. australis* (Ó Foighil & Smith, 1995). Polyploid Australian clones also bear a marked similarity in shell morphology to its sympatric sexual congener *L. australis*.

The large bulk of studied metazoan obligate asexual taxa were generated by interspecific hybridization producing asexual allopolyploid taxa (White, 1978; Vrijenhoek & Lerman, 1982; Vrijenhoek, 1989, 1994; Wetherington, Kotora & Vrijenhoek, 1987; Moritz, Wright & Brown, 1992) and a hybrid origin is likely for the highly heterozygous polyploid *Lasaea* clones. We had initially anticipated, therefore, that a subset of the Australian polyploid chromosome complement would reveal karyological affinities to that of *L. australis*. This is clearly not the case as there are no obvious karyological similarities among either of the three Australian congeners (Fig. 19).

Detailed genetic analyses of synthetic and naturally allopolyploid plant taxa have found that the hybrid genomes may undergo extremely rapid and dramatic rearrangements prior to stabilization (Bennett *et al.*, 1992; Kenton *et al.*, 1993; Song *et al.*, 1995). This may also be the case with polyploid *Lasaea* genomes and might account for the conspicuous lack of karyological concordance among the Australian taxa, and among other studied asexual congeners (Thiriot-Quiévreux *et al.*, 1988, 1989; Ó Foighil & Thiriot-Quiévreux, 1991). The hypothesis of an allopolyploid origin for Australian ploypoid clones could be tested by a molecular phylogenetic study of the three Australian congeners employing single copy nuclear genes. Additionally, the

hypothesis of radical karyological rearrangement in the Australian polyploid genomes could be tested by 'painting' polyploid metaphase spreads with labeled *L. australis* genomic DNA fragments to reveal regions of chromosomal homology. This technique is termed Genomic In-Situ Hybridization (GISH) and has been successfully employed to visualize the extensive post-hybridization karyological rearrangement of allopolyploid plant genomes (Bennett *et al.*, 1992; Kenton *et al.*, 1993).

Application of formal species names to asexual taxa is fraught with difficulty (Vrijenhoek, 1994; O'Hara, 1994) as they may contain a wide variety of clonal lineages that had independent origins. There is no consensus on this systematic dilemma. Some workers favour coining species names even in the absence of monophyly (Cole, 1985). Others recommend species names for each asexual lineage of independent origin (Frost & Wright, 1988; Echelle, 1990), a daunting objective in many genetically heterogeneous asexual taxa. When the parental species of a hybrid asexual lineage are known, a hyphenated biotype designation is phylogenetically very informative (Schultz, 1969; Vrijenhoek, 1994). Unfortunately this desirable latter option is unavailable for asexual *Lasaea* lineages. In our previous work on these organisms we have refrained from applying new species names to the large variety of highly diverse clonal lineages uncovered. Southeastern Australian polyploid *Lasaea* clones are morphologically and karyologically distinct from their two sympatric diploid congeners. However, in the absence of convincing evidence that they represent a monophyletic grouping, we consider it best not to apply a formal name to this assemblage at this stage.

In conclusion, three reproductively heterogeneous sympatric *Lasaea* congeners occur on southeastern Australian rocky shores and they provide a rare opportunity to explore the evolution of obligate asexuality, and its genetic and ecological consequences, in a marine metazoan taxon.

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APPENDIX. SPECIES DESCRIPTION OF *Lasaea colmani* sp. nov.

Minute, oval-subquadrate, thin-shelled, fragile, ≤ 1.4 mm in valve length. Posterior orthogyrate umbone is prominently inflated and globose in larger individuals. Shell margin is truncated posteriorally and, in larger individuals, has a non-convex ventral profile. External shell sculpture consists of microscopic pitting and individuals vary in color from pinkish-red to white with some shells possessing a thin brown periostracal layer comprised of commarginal threads. Hinge elements are weakly developed. The right valve contains a short anterior lateral tooth, an oblique resilium and two lamellar posterior lateral teeth. A single truncated anterior lateral tooth is present on the left hinge, together with a short thorn-like cardinal tooth, an oblique resilium and a lamellar posterior lateral tooth. The prodissoconch is non-umbonate and ≤ 40 µm in length. Direct developer with suprabranchial chamber brooding of young (3–12/brood) observed in specimens >0.8 mm in valve length. Ctenidia lack outer demibranchs. Meiotic and diploid with $2n = 40$.

The holotype is depicted in Figure 20 [Length = 1.1 mm, Height = 0.95 mm, Width (one valve) = 0.38 mm] and was deposited in the Australian Museum, Sydney (C.202742). Paratypes (4 specimens) were also placed in the Australian Museum (C.202743). Type Locality: Rocky shore at the headland of Long Reef Point, Sydney, New South Wales, Australia (151° 17′E, 33° 14′S). Collected in February 1992 among clumps of the tubiculous polychaete *Galeolaria caespitosa* in the mid-lower intertidal by D. Ó Foighil. The species name is given in honor of Philip Colman, who recently retired from the Australian Museum.

Lasaea colmani sp. nov. can be readily distinguished from sympatric Australian congeners using morphological, developmental and genetic characters. Adults of the new species differ, in having an inflated orthogyrate umbone, oval-subquadrate outline, truncated posterior margin, non-convex ventral margin and much smaller body size than their much larger, progyrate, suboval sympatric congeners which have convex ventral margins (Fig. 21). Prodissoconch shape and size easily distinguish juveniles,

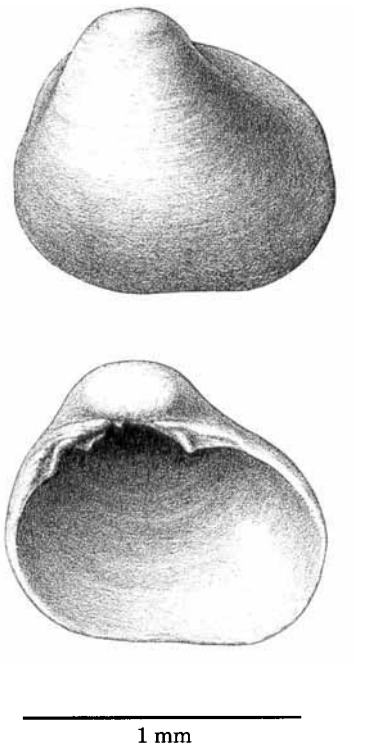

Figure 20. *L. colmani* sp. nov. Holotype (Australian Museum, C. 202742), showing (above) exterior view of right valve and (below) interior view of left valve.

and adults, of all three sympatric Australian congeners: *Lasaea colmani* sp. nov. (non-umbonate, D-shaped, 300–340 μm in length); *L. australis* (umbonate, 220–300 μm in length); unnamed sympatric polyploid congener (non-umbonate, D-shaped, 420–480 μm in length) and represent the most reliable character for distinguishing larger specimens of the polyploid taxon from *L. australis*. The new species also differs in lacking an outer demibranch, present in the sympatric congeners, in its ctenidia. It is diploid with $2n=40$, whereas the only other known diploid congener, *L. australis*, has a smaller chromosome complement ($2n=36$), (Thiriot-Quiévreux, 1992). Molecular phylogenetic analyses have revealed the presence of two major mitochondrial clades in the genus *Lasaea*, each of which has a global distribution (Ó Foighil & Smith 1995, 1996). *Lasaea colmani* spec. nov. occurs in a separate clade to its sympatric Australian congeners but together with clonal polyploid lineages from the northeast Pacific, Kerguelen Island and the northeast Atlantic (Ó Foighil & Smith 1995, 1996).

Lasaea colmani sp. nov. is easily distinguished from studied global direct-developing populations of this genus by its prominently inflated orthogyrate umbone, exceptionally small adult size and diploid condition. Ongoing global molecular phylogenetic characterization of the genus *Lasaea* has failed to find close relatives of this species among Australian, New Zealand, Japanese, Kerguelen Island, South African, European, Floridian and West Coast North American samples (Ó Foighil & Smith 1995, 1996; Taylor & Ó Foighil, 2000). *Lasaea colmani* sp. nov. is not unique among its congeners in lacking an outer demibranch, as Ponder (1971) has also reported this condition in the New Zealand species *Lasaea maoria* (Powell, 1933), (type location = Rangitoto Island, Auckland). *L. maoria* is readily distinguished from *Lasaea colmani* sp. nov. based on its moderately inflated and more centrally placed umbone (Fig. 21), convex ventral shell margin in adults, absence of significant red pigmentation and much greater body size.

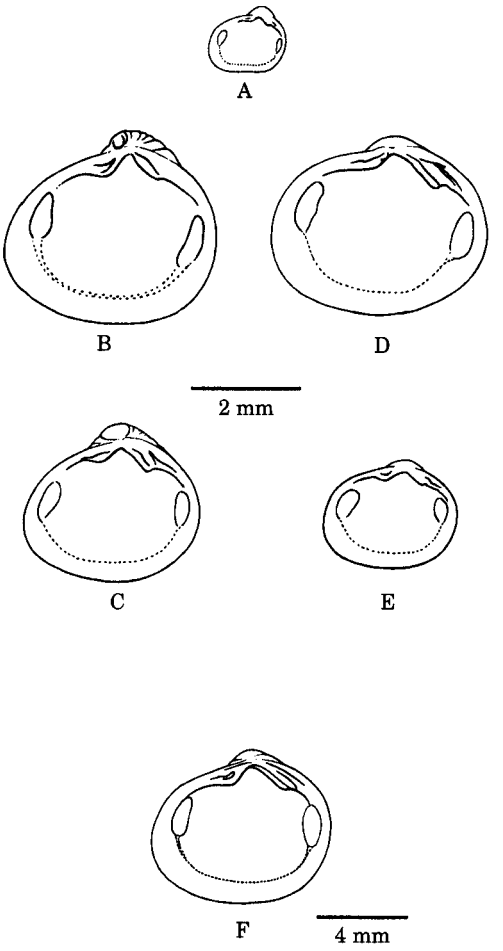

Figure 21. Outlines of the right valves of *Lasaea* taxa from the Sydney, Australia study area (A–C), New Zealand (D, E), and Timor (F). (A) *L. colmani* sp. nov.; (B) *L. australis*; (C) polyploid Australian clonal lineage; (D) holotype of *L. rubra hinemoa* Finlay, 1928 (AK 70387); (E) holotype of *L. maoria* (AK 70375); (F) syntype of *L. australis* (MNHN). Scale bar: A–E = 2 mm; F = 4 mm. *L. colmani* sp. nov. is clearly distinguishable by its posterior inflated orthogyrate umbone, non-convex ventral margin and much smaller size.