**Supporting Information Figure Legends**

**Fig. S1.**

The identification of genes isolated from *Prionocidaris baculosa*. Alignments of deduced amino acid sequences and construction of phylogenetic trees were performed using MacClade 4.08 (http://macclade.org/macclade.html) and Clustal X (http://www.clustal.org/clustal2/) on the sequences listed below: the sequence of bHLH for hesC, full-length sequence for alx1, T-box sequence for tbr, C-terminal 74 amino acid sequence of the Ets domain for ets1, C-terminal 108 amino acid sequence of Gcm domain for gcm, and C-terminal 83 amino acid sequence of peptidyl-prolyl cis-trans isomerase domain for cyp1 were used. (A) Identification of *PbhesC*. The phylogenetic tree suggests the isolated gene belongs to the HES family but not the HERP family. In the echinoderm *hesC* group (green box) of the HES family, the *PbhesC* candidate is closely related to the other echinoid *hesC* genes rather than the asteroid *hesC*. (B) Identification of *Pbalx1*. The tree clearly supports that the isolated sequence is included in the Cart1 group. Cart1 genes were divided into 2 groups: genes from vertebrates and *alx* genes of echinoids. *Pbalx1* was in the group of the echinoid *alx1* genes (blue box) but not in *alx4*. (C) Identification of *Pbtbr*. Analysis using 3 subfamily genes of the T-box family (*Tbr*, *Bra*, and *Tbx*) supported the theory that the isolated gene belongs to the echinoderm *Tbr* family (green box). In echinoderm *tbr* genes, our candidate sequence was separated into echinoid *tbr* (blue box) and asteroid *tbr*. (D) Identification of *Pbgcm*. The tree shows that the *gcm* family genes were classified into genes from echinoderms (green box) and the other animals. Among echinoderm genes, the isolated gene showed a higher homology with the other echinoid *gcm* genes (blue box) than with the asteroid *gcm*. (E) Identification of *Pbcyp1*. The

phylogenetic tree analyzed with 8 *cyclophillin* genes identified in *Strongylocentrotus purpuratus* suggests that *Spcyclophilin1* shows the highest homology to the isolated gene (bootstrap support 100%). (F) Identification of *Pbets1*. The C-terminal 74 amino acid sequence of the isolated gene was compared with those of the other *ets* family genes. Our sequence is 100% identical to those deduced from echinoid *ets1* genes (*Smets1, PlETS1, and Spets1*) and is identical, except for 1 amino acid, with that of asteroid *ets1* ortholog. In contrast, there were a number of differences in the amino acid residues compared with the sequences of other *ets* family genes identified in *S. purpuratus* (*Sp-Pea, Sperg, and Sp-Gabp*). Accession numbers are as follows: Sp-HesC, SPU_021608; HphesC, AU274707; SmhesC, AB569638; PmHesC, GU251976; SpHes, AY445629; Sk-hes1, NM_001164994; Dmh, NM_001014577; Sp-Hairy, SPU_006813; Sp-Hey4, SPU_015712; DmE(spl)-HLH-mdelta, X67048; DmHesr-1, AF151523; Sp-Hey, SPU_009465; HsHEY1, NM_012258; SpAlx1, NM_214644; LvAlx1, AY277400; PlAlx1, DQ536192; Smalx1, AB569635; Sp-Alx4, SPU_22816; MmAlx1, NM_172553; Drcart1, GU056833; MmAlx3, NM_007441; MmAlx4, NM_007442; Dmal, NM_164382; Drarx, NM_131384; MmArx, NM_007492; Sp-Arx, XM_001189711; PlSKE-T, AJ309216; HpTb, EF530737; Pjtbr, FJ715949; Smtbr, FJ714958; Ap-T-brain, AB032259; Sktbr1, NM_001164990; MmEomes, NM_010136; MmTbr1, NM_009322; MmTbx6, AY654733; Sp-Tbx-2-3, SPU_023386; MmTbx2, AF244917; MmTbx1, AF349658; Sp-Bra, SPU_013015; MmT, NM_009309; Pmgcm, HP129833; LvGCM; EU306538; Spgcm; NM_214661; BfGcm; XM_002591735; Dmgcm; U34039; MmGcm2; NM_008104; MmGcm1; NM_008103; Spcyclophilin1, NM_0016033647; Sp-Ppib, SPU_013756; Sp-Pdpi (cyclophilin) L7, SPU_008305; Sp-Pdpi (cyclophilin) L5, SPU_000637; Sp-Pdpi (cyclophilin) L8, SPU_028896; Sp-Ppil4; SPU_004626; Sp-Pdpi (cyclophilin) L6, SPU_015088; Sp-Pdpi (cyclophilin) L9, 022479;

Smets1; AB569636; PlEts1; AY442298; SpEts1; NM_214533; ApEts; AB569245; Sp-Pea; SPU_014576; SpErg; AY508725; and Sp-Gapb; SPU_021557.

**Fig. S2.**

Spatial gene expression analysis at the mid-blastula, mid-gastrula, and pluteus stages and quantitative expression analysis through early development of the *P. baculosa* embryo. (A1–G1 and H) Mid-blastulae at 12 h postfertilization (hpf). (A2–G2) Mid-gastrulae at 25 hpf. (A3–G3) Pluteus larvae at 72 hpf. (A1–A3) Embryonic development. (A1 and A3) Living embryos. (A2) Immunohistochemistry was performed on the fixed embryo with a P4 antibody. The expression of P4 is observed in the mesenchyme cells ingressed into the blastocoel. (B1–G1, B2–G2, B3–G3, and H) The embryo analyzed by whole-mount *in situ* hybridization (WMISH). Scale bar = 50 μm. (B4–G4) Transcript levels analyzed by quantitative real-time polymerase chain reaction (QPCR) from the unfertilized egg (0 hpf) to the pluteus larva (72 hpf) stages. The X-axis shows the time after fertilization. The Y-axis shows the relative amount of subjected mRNA to *PbmitCOI* mRNA. The error bars show standard deviations. (I) Table showing the onset of *Pbalx1*, *Pbtbr*, *Pbets1*, and *Pbgcm* expression during the early embryonic stages. The number of embryos with localized gene expression was counted at 4 stages: 64-cell (6 hpf), 120-cell (8 hpf), 240-cell (10 hpf), and 420-cell (12 hpf) stages. Hpf: hours postfertilization. ND: not determined.

**[Transition of mRNA expression level through early development]**

For all genes other than *Pbalx1*, maternal mRNA is detected. (B4) Expression of *PbhesC* continues from the egg (0 hpf) until the pluteus larva (72 hpf) stage. (C4–F4) The expression levels of *Pbalx1*, *Pbtbr*, *Pbets1*, and *Pbgcm* increased during the blastula stages and reached a peak at the gastrula

stage (25 hpf or 36 hpf). (G4) The expression level of *Pbcyp1* markedly increased at 25 hpf and reached a peak at 36 hpf.

**[The onset of region-specific expression]**

Strong expression regions of *PbhesC* can be observed as spots in whole embryos by the 120-cell stage (8 hpf). (I) Localized expression of *Pbalx1* and *Pbets1* starts at the 120-cell stage (8 hpf), whereas that of *Pbtbr* and *Pbgcm* starts at the 240-cell stage (10 hpf) at 1 pole of the embryo. As mentioned below, region-specific expression of *Pbcyp1* starts from the mid-gastrula stage.

**[The expression pattern at mid-blastula stage (12 hpf)]**

(B1) There are a number of spots expressing *PbhesC* at this stage. To examine the relationship between the location of patches and the animal–vegetal axis, double-WMISH with the vegetal pole marker *Pbwnt8* (Yamazaki et al., 2012) was performed. In the double-stained embryos, 1 of the patches of *PbhesC* expression (arrowhead) was always observed in the center of the *Pbwnt8*-expressing area (orange double-headed arrow), suggesting that the vegetal pole cells express *PbhesC*. (C1–F1) The expression regions of *Pbalx1* (C1), *Pbtbr* (D1), *Pbets1* (E1), and *Pbgcm* (F1) are located at 1 pole of the mid-blastula. The multiplex-WMISH using RNA probes of *wnt8*, *alx1*, *tbr*, *ets1*, and *gcm* (H). All the embryos analyzed had 1 spot, which included *alx1*, *tbr*, *ets1*, and *gcm* expression, located in the center of the *wnt8*-expressing region (orange double-headed arrow in H), suggesting their vegetal expression. (G1) *Pbcyp1* does not show obvious expression.

**[The expression pattern at the mid-gastrula stage (25 hpf)]**

(B2–G2) The expression of all genes is observed in the archenteron (bracket) and/or the ingressed mesenchyme cells. Four genes other than *PbhesC* and *Pbgcm* are expressed in the ingressed skeletogenic mesenchyme cells. White arrowheads in (B2) and (F2) indicate mesenchyme cells with

no gene expression. In the archenteron, the lower part expresses *PbhesC* (B2), the middle part expresses *Pbgcm* (F2), and the upper regions shows expression of *Pbtbr*, *Pbets1*, and *Pbcyp1* (D2, E2, and G2).

**[The expression pattern in the pluteus larva at 72 hpf]**

(B3–G3) The obvious expression of all genes other than *Pbtbr* is detected at this stage. (B3) Expression of *PbhesC* is observed in the tip of the archenteron (arrow) and the coelomic pouch (arrowhead) as well as the ectodermal region. (C3) The pluteus larva weakly expresses *Pbalx1* in the mesenchyme cells positioned along the postoral rods. (E3) *Pbets1* is expressed in both the ectoderm and the mesenchyme cells located in the tip of the postoral arms (double-headed arrows). (F3) The *Pbgcm*-expressing cells are located in the tip of the archenteron, the coelomic pouches (arrowhead), or the ectoderm (arrow). (G3) Expression of *Pbcyp1* is detected in the coelomic pouches (arrows) and in mesenchyme cells at the tip of the postoral rods (arrowheads) as well as some other regions.

**Fig. S3.**

QPCR analysis of *hesC* mRNA in hesC perturbed embryos of *P. baculosa* (A), *S. mirabilis* (B), and *H. pulcherrimus* (C). Analyses are performed at the blastula stage. The hpf is indicated in the upper left corner of each graph. The Y-axis shows the cycle difference (Ct) in QPCR when compared with the uninjected control. The results from 2 batches are shown. Error bars are standard deviations. In all cases, the amount of *hesC* mRNA is significantly increased by species-specific hesC-MO, whereas the MO derived from the sequence of another species shows no effect.

**Figure S1.**

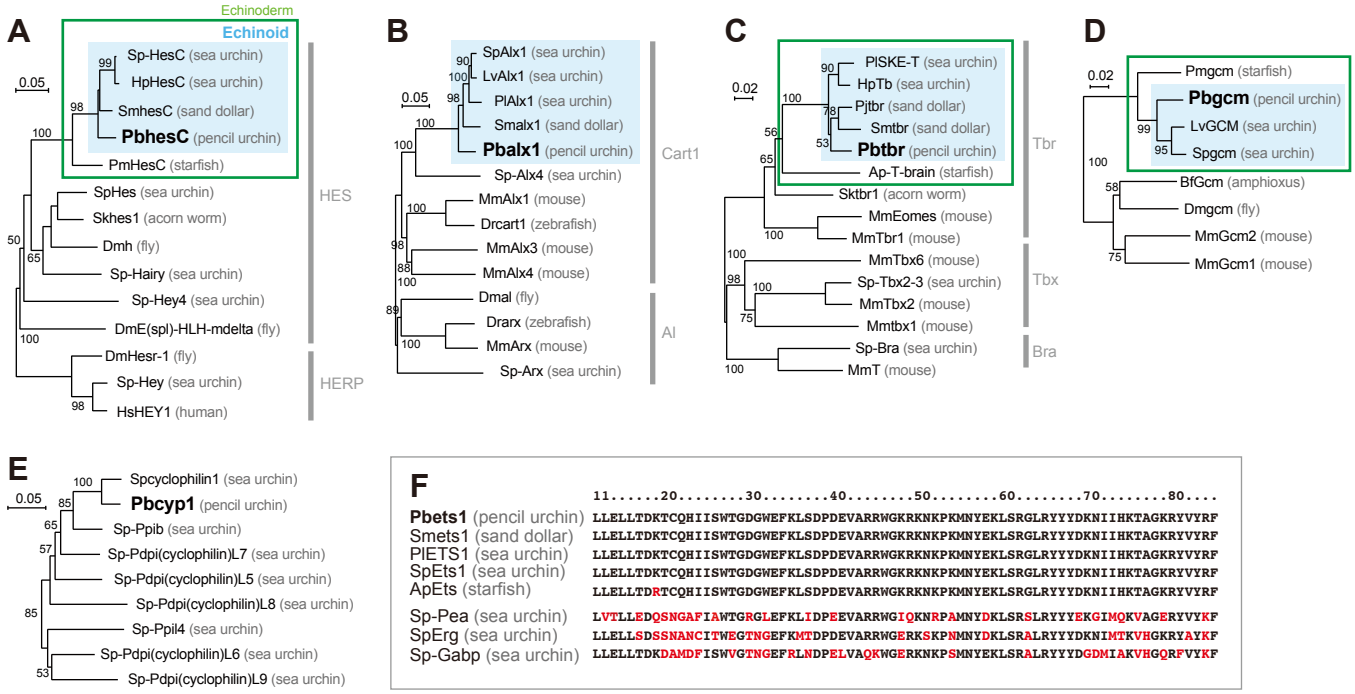
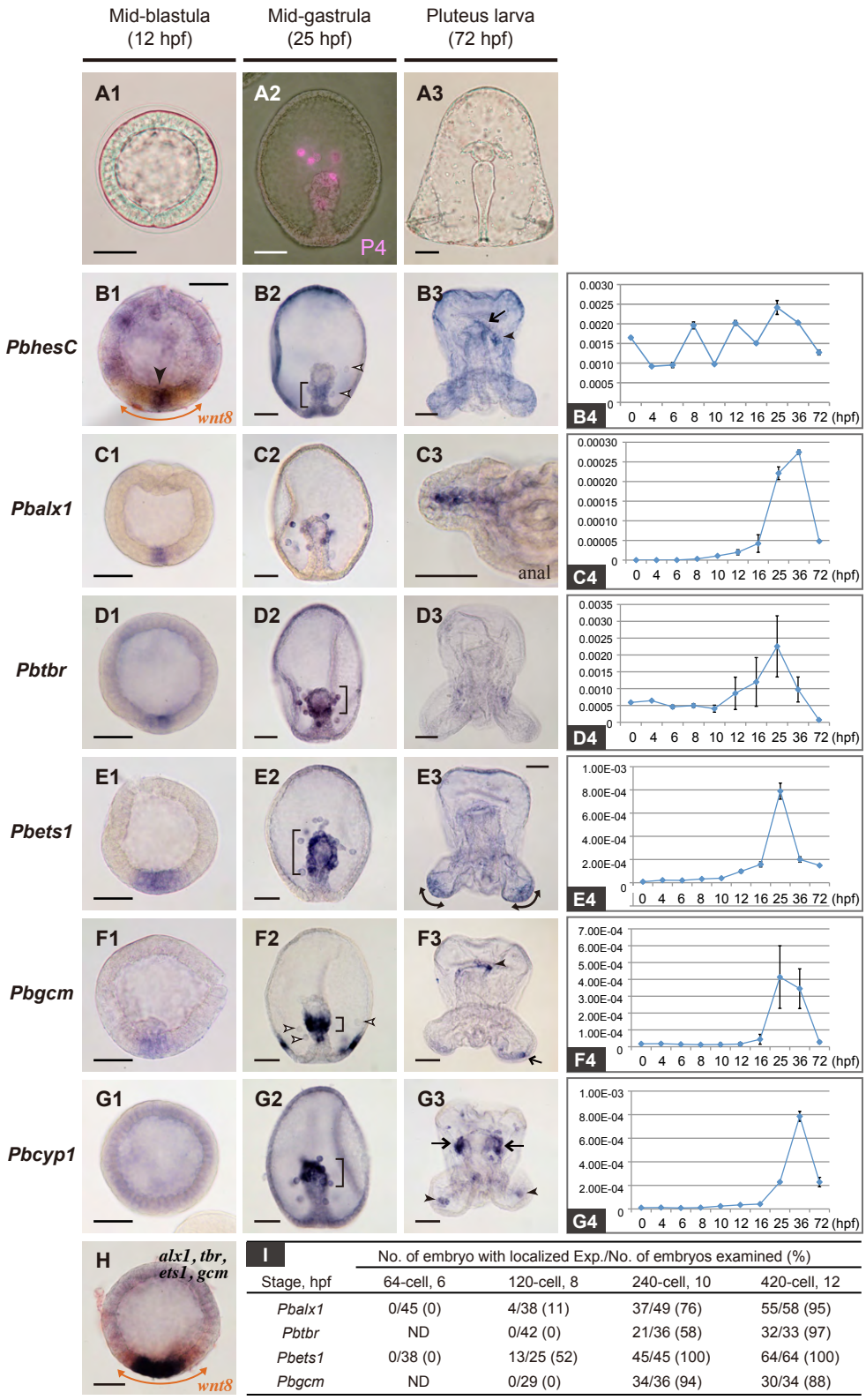

A
Echinoderm
Echinoid
Sp-HesC (sea urchin)
HpHesC (sea urchin)
SmhesC (sand dollar)
PbhesC (pencil urchin)
PmHesC (starfish)
SpHes (sea urchin)
Skhes1 (acorn worm)
Dmh (fly)
Sp-Hairy (sea urchin)
Sp-Hey4 (sea urchin)
DmE(spl)-HLH-mdelta (fly)
DmHesr-1 (fly)
Sp-Hey (sea urchin)
HsHEY1 (human)
HES
HERP
B
SpAlx1 (sea urchin)
LvAlx1 (sea urchin)
PlAlx1 (sea urchin)
Smalx1 (sand dollar)
Pbalx1 (pencil urchin)
Sp-Alx4 (sea urchin)
MmAlx1 (mouse)
Drcart1 (zebrafish)
MmAlx3 (mouse)
MmAlx4 (mouse)
Dmal (fly)
Drarx (zebrafish)
MmArx (mouse)
Sp-Arx (sea urchin)
Cart1
Al
C
PlSKE-T (sea urchin)
HpTb (sea urchin)
Pjtbr (sand dollar)
Smtbr (sand dollar)
Pbtbr (pencil urchin)
Ap-T-brain (starfish)
Sktbr1 (acorn worm)
MmEomes (mouse)
MmTbr1 (mouse)
MmTbx6 (mouse)
Sp-Tbx2-3 (sea urchin)
MmTbx2 (mouse)
Mmtbx1 (mouse)
Sp-Bra (sea urchin)
MmT (mouse)
Tbr
Tbx
Bra
D
Pmgcm (starfish)
Pbgcm (pencil urchin)
LvGCM (sea urchin)
Spgcm (sea urchin)
BfGcm (amphioxus)
Dmgcm (fly)
MmGcm2 (mouse)
MmGcm1 (mouse)
E
Spcyclophilin1 (sea urchin)
Pbcyp1 (pencil urchin)
Sp-Ppib (sea urchin)
Sp-Pdpi(cyclophilin)L7 (sea urchin)
Sp-Pdpi(cyclophilin)L5 (sea urchin)
Sp-Pdpi(cyclophilin)L8 (sea urchin)
Sp-Ppil4 (sea urchin)
Sp-Pdpi(cyclophilin)L6 (sea urchin)
Sp-Pdpi(cyclophilin)L9 (sea urchin)
F
11.....20.....30.....40.....50.....60.....70.....80....
Pbets1 (pencil urchin) LLELLTDKTCQHIISWTGDGWEFKLSDPDEVARRWGKRKNKPKMNYEKLSRGLRYYYDKNIIHKTAGKRYVYRF
Smets1 (sand dollar) LLELLTDKTCQHIISWTGDGWEFKLSDPDEVARRWGKRKNKPKMNYEKLSRGLRYYYDKNIIHKTAGKRYVYRF
PlETS1 (sea urchin) LLELLTDKTCQHIISWTGDGWEFKLSDPDEVARRWGKRKNKPKMNYEKLSRGLRYYYDKNIIHKTAGKRYVYRF
SpEts1 (sea urchin) LLELLTDKTCQHIISWTGDGWEFKLSDPDEVARRWGKRKNKPKMNYEKLSRGLRYYYDKNIIHKTAGKRYVYRF
ApEts (starfish) LLELLTDRTCQHIISWTGDGWEFKLSDPDEVARRWGKRKNKPKMNYEKLSRGLRYYYDKNIIHKTAGKRYVYRF
Sp-Pea (sea urchin) LVTLLEDQSNGAFIAWTGRGLEFKLIDPEEVARRWGIQKNRPAMNYDKLSRSLRYYYEKGIMQKVAGERVVYKF
SpErg (sea urchin) LLELLSDSSNANCITWEGTNGEFKMTDPDEVARRWGERKSKPNMNYDKLSRALRYYYDKNIMTKVHGKRYAYKF
Sp-Gabp (sea urchin) LLELLTDKDAMDFISWVGTNGEFRLNDPELVAQKWGERKNKPSMNYEKLSRALRYYYDGDMIAKVHGQRFVYKF

**Figure S2.**




**I**

| Stage, hpf | No. of embryo with localized Exp./No. of embryos examined (%) | | | |
|---|---|---|---|---|
| | 64-cell, 6 | 120-cell, 8 | 240-cell, 10 | 420-cell, 12 |
| *Pbalx1* | 0/45 (0) | 4/38 (11) | 37/49 (76) | 55/58 (95) |
| *Pbtbr* | ND | 0/42 (0) | 21/36 (58) | 32/33 (97) |
| *Pbets1* | 0/38 (0) | 13/25 (52) | 45/45 (100) | 64/64 (100) |
| *Pbgcm* | ND | 0/29 (0) | 34/36 (94) | 30/34 (88) |

**Figure S3.**

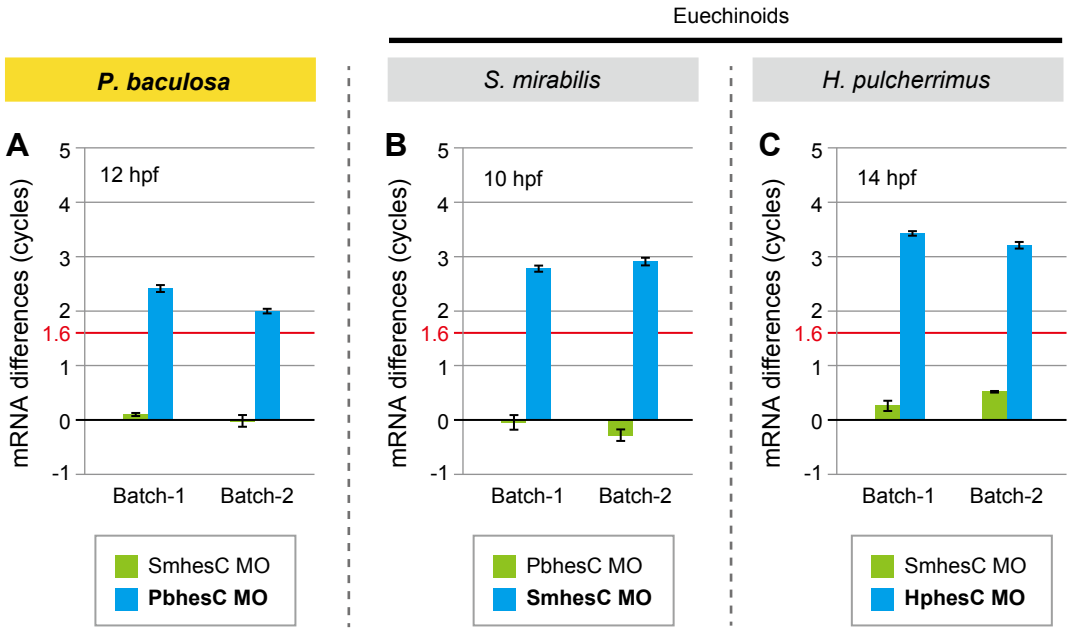

Euechinoids
P. baculosa
S. mirabilis
H. pulcherrimus
A
B
C
12 hpf
10 hpf
14 hpf
mRNA differences (cycles)
5
4
3
2
1.6
1
0
-1
Batch-1
Batch-2
SmhesC MO
PbhesC MO
PbhesC MO
SmhesC MO
SmhesC MO
HphesC MO

**Table S1.** Transition of total cell numbers during early development

| Hpf | 5 | 6 | 8 | 10 | 12 | 16 |
|---|---|---|---|---|---|---|
| Average ± | 31.8 ± | 61.5 ± | 128.9 ± | 246.4 ± | 449.8 ± | 556.6 ± |
| SD | 1.8 | 1.5 | 28 | 46 | 49 | 56 |
| Range | 30–32 | 61–62 | 120–147 | 232–272 | 423–470 | 495–624 |

**Hpf:** hours postfertilization.

**Average:** the average of total cell numbers from 10 individuals.

**SD:** standard deviation.

**Range:** from the minimum number to the maximum number.

**Table S2.** Primers used in the present study.

| Gene | Utilize | Primer name | Sequence 5′–3′ |
|---|---|---|---|
| *PbhesC* | degenerate PCR | F | AARATGGARAARGCTGAYAT |
| | | R | HGCANCGRTCNGCNAGRTG |
| | RACE | F1 | GACAGTGCGTTACTTGAAAGAGCTCCAG |
| | | F2 | AGTCGAGTGAGGGCAGCAGACCGTTGT |
| | | R1 | TGTCGAGGTCGATACTCTCGCAGGATGA |
| | | R2 | GCTGGAGACCTCACTCAAACATTCGGTGAAG |
| | QPCR | F | CACATGCAGTCCAGGCAGTT |
| | | R | GCCTGGCGAGGAAAGACTAA |
| *Pbalx1* | degenerate PCR | F | AAARMGNMGNAAYMGNACNACNTT |
| | | R | ATANCCRTTCATCATNCCNAYNAC |
| | RACE | F1 | CACGAGTTATCAGCTGGAGGAAATGGAGAAG |
| | | F2 | TCCAGACGTATATTGCAGAGAACAACTC |
| | | R1 | TGGCCTCTGTTAGGTCACATCTGAGAGC |
| | | R2 | GAGTTGTTCTCTGCAATATACGTCTGGAT |
| | QPCR | F | GTGAGCGGTTCCAGCAGTTT |
| | | R | CTGGTGGTTGATGCGTGTCT |
| *Pbtbr* | RACE | F1 | CGCTCCAGTACAACGTGTTTGTCGATATG |
| | | F2 | GGTCCCATGTGGCCAGGCTGAGAAT |
| | | R1 | CTGCGTCTCGGGAAAGCTATGGGTCTGAA |
| | | R2 | CTAACACATGGATGCGAGGTTGGTACTTG |
| | QPCR | F | CAATCATCGAGGGAAGGACAA |
| | | R | GGATTCTTCGGTCGCTCAGT |
| *Pbets1* | RACE | F1 | ACATGTCAGCACATCATCAGCTGGACC |
| | | F2 | AGTTCAAGCTCTCCGACCCGGACGAA |
| | QPCR | F | GGCAAGCGCAAGAACAAAC |
| | | R | CTGGAGATCGCACACGAATC |
| *Pbgcm* | degenerate PCR | F | GGGNTGGGCNATGMGNAAYAC |
| | | R | CCARAARTGNGTNACNGGRTA |
| | RACE | F1 | AGGTGTTTTCGTTTGCTCCAACAACTGTCA |

| | | | |
|---|---|---|---|
| | | F2 | ACATTGTCACCGTTCGACCGGCCACGT |
| | QPCR | F | ACGCTTCAAGCCAAGACGAT |
| | | R | TGCCGAAGGAAGCATAACTG |
| *Pbcyp1* | degenerate PCR | F | CAARTTYCAYMGNGTNAT |
| | | R | CTTNCCRWANACNACRTG |
| | RACE | F1 | GATTCAAGGCGGTGACTTTGTCTCAGTAG |
| | | F2 | GAGGATGAGAACTTCAAGCTGGACCACTA |
| | QPCR | F | CCAACGGTTGCCAGTTCTAC |
| | | F | TTCAATGGCCTTCACCACAT |
| *PbmitCO1* | QPCR | F | CGGAATGGTTTATGCGATGA |
| | | R | TCCGGTTGGAACTGCTATGA |
| *SmmitCO1* | QPCR | F | TTCCTAGTTTGAGCCCACCAT |
| | | R | AGTGGGAACGGCGATTATCA |
| *SmhesC* | QPCR | F | ATGACGGTGCGTTACCTGAA |
| | | R | TTCTCGCACGATGACATGAA |
| *HpmitCO1* | QPCR | F | CCGCATTCTTGCTCCTTCTT |
| | | R | TGCTGGGTCGAAGAAAGTTG |
| *HphesC* | QPCR | F | TGTGCCATCACCAGTCACAG |
| | | R | GTGTTGGCTGGAAGGAGGAG |